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ALKALOIDS OF *SANGUINARIA CANADENSIS*

by

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A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies for acceptance, a thesis entitled "Alkaloids of Sanguinaria Canadensis" submitted by Reginald Frank Chandler in partial fulfillment of the requirements for the degree of Master of Science.

ABSTRACT

In a survey of the alkaloids of Sanguinaria canadensis previously described, it was found that doubt existed as to what alkaloids were actually present.

The present investigation of the alkaloids of this plant resulted in the isolation of eleven alkaloids and the detection of at least six others. Two of the alkaloids extracted were previously unreported benzophenanthridine alkaloids. Each of these new alkaloids, which were named methoxychelerythrine and sanguinaricine, has five alkoxy substituents. This was the first time that dihydrosanguinarine, dihydrochelerythrine, oxychelerythrine, oxymethoxychelerythrine and oxysanguinaricine have been isolated from this plant.

ACKNOWLEDGEMENTS

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Finally, the author wishes to express appreciation to his wife, who did the typing, and family for their encouragement and assistance in the preparation of the manuscript.

DEDICATION

This thesis is dedicated to my loving mother, whose foresight and encouragement inspired me initially to undertake graduate work and who was a main-stay until her untimely death midway through my program.

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PART I

INTRODUCTION AND STATEMENT OF PROBLEM

Bloodroot, Sanguinaria canadensis, genus Papaveraceae (1,2), is a small perennial herb native to Eastern North America. It is found in Canada mainly on the eastern seaboard and in Ontario. The plant derives its name from its latex, which turns blood-red upon exposure to air. The dried rhizome of bloodroot has been used by the indigenous people of North America in their medical practice (2,3) and is still in use today in Compound White Pine Syrup (4) and as a component in Pinocodeine (Charles E. Frosst and Company), despite the recommendation by Boyd and Palmer (5) that it be deleted from pharmaceuticals because it produces its effect by gastric irritation. Through the years, the plant extract has been used as an expectorant (2,3,4,6,7,8), an antibacterial (9), as a smooth muscle stimulant (10), as a local anesthetic (3,11), in atonic dyspepsia (6,7,12) and to stimulate respiration (3). It is sternutatory and it stimulates the flow of saliva as well as increasing intestinal peristalsis (3). It is also said to possess a colchicine-like effect of doubling the chromosomes in cells (8,13,14,15) and has been used experimentally in treatment of ascites tumor cells (14).

Besides these medicinal uses, sanguinarine and chelerythrine were found to be the most effective of sixty-two alkaloids in completely inhibiting the root-rot fungus Phymatotrichum omnivorum (17,18).

In 1829, Dana (19) isolated an alkaloid from bloodroot which he called sanguinarine, due, no doubt, to the color of its easily formed salts. It was not, however, until 1924 that a pure sample of the base was obtained (20). The structure for sanguinarine, (I, TABLE I), was established by von Bruchhausen and Bersch (21,22) in 1930 and confirmed by Späth and Kuffner (23) in 1931. Although chelerythrine, (II, TABLE I), was origionally isolated from Chelidonium majus by Probst (24) in 1839, its first reported occurrence in bloodroot was by König in 1891 (25).

It is a contaminant of commercial sanguinarine and can only be removed from it by a complicated fractionation procedure (26,27).

Protopine, (I, TABLE II), and α-allocryptopine, (II, TABLE II), have also been isolated from an alcoholic extract of the plant (27,28,29,30).

Until 1953, chelerythrine and sanguinarine were thought to be the only benzophenanthridine alkaloids present in S. canadensis although other alkaloids were reported present (27,28,29,31,32). The most recent investigation of bloodroot (28) reported finding eleven alkaloids present, seven of which are new. Four of these new alkaloids, chelirubrine, chelilutine, sanguirubrine and sanguilutine, although ill defined, were thought to be benzophenanthridine alkaloids but no proof was advanced by the author for his belief. Besides these, berberine, coptisine, and oxy-sanguinarine were also reported (28).

It seemed of interest, therefore, to reinvestigate the alkaloidal constituents of Sanguinaria canadensis to confirm or refute the presence of these minor compounds.

PART II

LITERATURE SURVEY

A. GENERAL

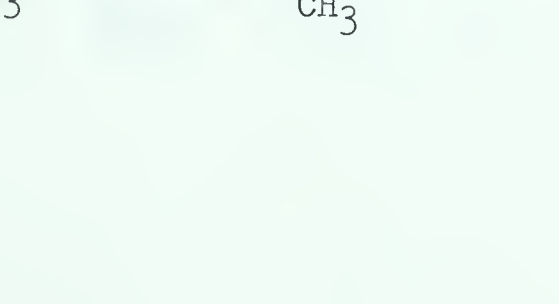
The four major alkaloids previously isolated from bloodroot belong, chemically, to two different groups of alkaloids. Protopine and α-allocryptopine belong to the protopine group (30,33,34) and sanguinarine and chelerythrine to the 1,2-benzophenanthridine [benzo (c) phenanthridine, α-naphthaphenanthridine] group.

Although the protopine group of alkaloids has been found extensively in nature and their structures established, only seven members of the benzophenanthridine group have been identified; two of which, xanthofagarine (35,36) and avicine (37), were reported in the last five years.

The physical properties of these bases are summarized in tables I, II and III.

TABLE I

1,2-BENZOPHENANTHRIDINE ALKALOIDS

NO.		M.p. °C.	DERIVATIVE	M.p. °C.	REFERENCE
I	SANGUINARINE	240			20,26,28,38,39,40
	(ψ-CHELERYTHRINE)	213			25,27
	$C_{20}H_{14}O_4N$	195-7			20
			ψ-CN	237-8	20,26
				242-3	28
			ACETATE	225	38
			HCl	272-3	28
			NOR-	279-80	28,38,41
			OXY-	360-61	26,28,39,42,43
			DIHYDRO-	188-89	26,42
II	CHELERYTHRINE	210			26
	$C_{21}H_{18}O_4N$	282-3			28
			ψ-CN	258-60	20,28,26,41,44
			HNO_3	240	45
			HCl	213-4	28
			HI	150-60	46
			AURICHLORIDE	233	39
			9-Et ether	239-42	45
			9-Et ether ψ-CN	229-33	45
			NOR-	221-2	41,45
			OXY-	199-201	45
			DIHYDRO-	166-7	26,42,47
III	XANTHOFAGARINE	278-82			36,48
	(NITIDINE)		ψCN	234	35
	$C_{21}H_{18}O_4N$		ACETATE	255-60	35
			HCl	285-6	35
			NOR-	277-8	36,49
			OXY-	284-5	35
			DIHYDRO-	221-3	35
				208-11	50
			DIHYDRO-HCl	215-6	35
			DIHYDRO-MeI	268	35

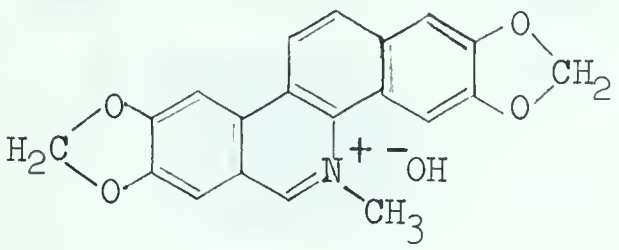
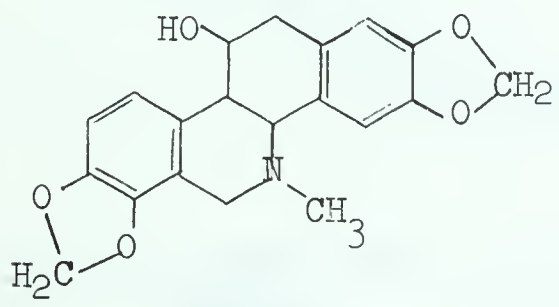
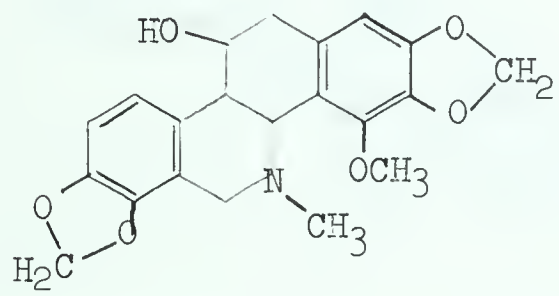
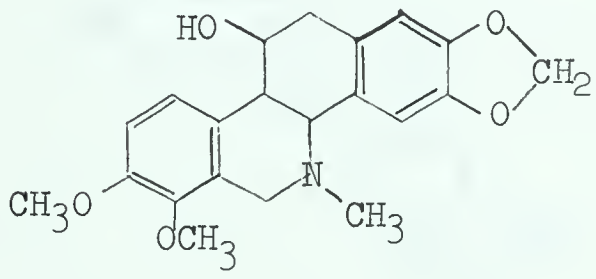
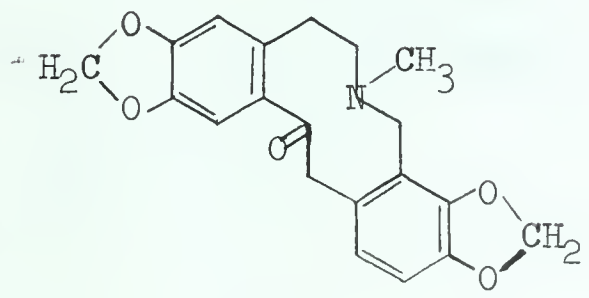
NO.		M.p. °C.	DERIVATIVE	M.p. °C.	REFERENCE
IV	AVICINE $C_{20}H_{14}O_4N$	NONE REPORTED			
			ψ-CN	340	37
			ACETATE	160	37
			OXY-	275-77	37
			DIHYDRO-	211-13	37
			DIHYDRO-HCl	255-58	37
V	CHELIDONINE $C_{20}H_{19}O_5N$	135-36 dl-216			26,51,52 49
			ψ-CN	194-95	26,53
			ACETATE	161	54
			HCl	204-05	26,55
			NOR-	199	55,56
			N-ACETYL-	152	57
			N-ACETYLNOR-	195-96	55,56
			OXY-	285	20,26
			AURICHLORIDE	155	23
			CHLOROPLATINATE	200	23
VI	METHOXY- CHELIDONINE $C_{21}H_{21}O_6N$	221			20,26
			AURICHLORIDE	237-38	20,26
			O-ACETYL-	147	20
			O-ACETYL-CHLOROACETATE	136-37	20
VII	α-HOMO- CHELIDONINE $C_{21}H_{23}O_5N$	182			26,58
					

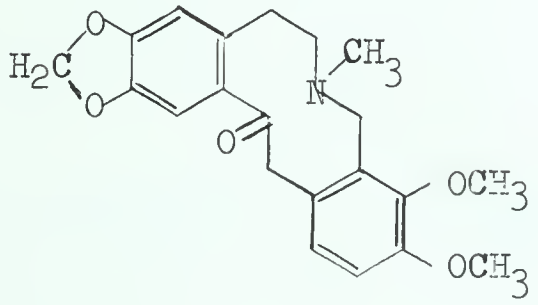
TABLE II

ASSOCIATED ALKALOIDS

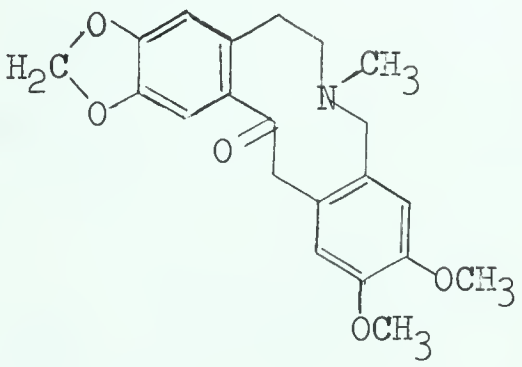
NO.		M.p. °C.	DERIVATIVE	M.p. °C.	REFERENCE
I	PROTOPINE (FUMARINE, MACLEYINE) $C_{20}H_{19}O_5N$	207			34,59
			AURICHLORIDE	198	60
			BENZOATE	217	60
			PICRATE	248	20



II	α-ALLOCRYPTOPINE (β-HOMO-CHELIDONINE α-FAGARINE) $C_{21}H_{23}O_5N$	160&170			61
			AURICHLORIDE	187	52



III	FAGARINE II $C_{21}H_{23}O_5N$	198-99			62,82
			HCl	200-02	62,82
			HBr	208-10	62,82
			MeI	234	62,82
			PICRATE	214	62,82
			AURICHLORIDE	218-19	82



NO.		M.p. °C.	DERIVATIVE	M.p. °C.	REFERENCE
IV	BERBERINE	160			63;64
	$C_{20}H_{19}O_5N$	145			60

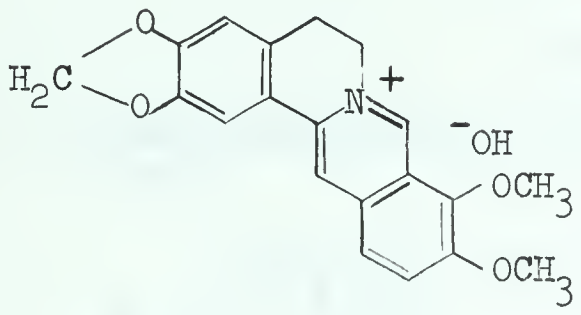


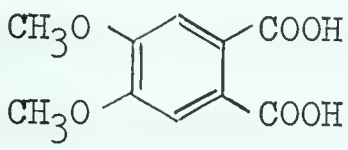
TABLE III

DEGRADATION AND REACTION PRODUCTS OF

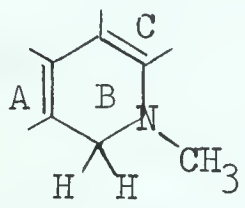
1,2-BENZOPHENANTHRIDINE ALKALOIDS

NO.		M.p. °C.	DERIVATIVE	M.p. °C.	REFERENCE
I	1,2-BENZOPHENANTHRIDINE $C_{17}H_{11}N$	135.5			23,65,66
			HCl	235	65
			PICRATE	256	65
			OTHERS	...	35,37,41, 49, 50,67-76
II	HYDRASTIC ACID $C_9H_6O_6$	172-75 187-88			77,78 77,79
			ANHYDRIDE	175	79
			IMIDE	275-77	79
				290-91	80
			DIMETHYL ESTER	88-89	79
			N-METHYLIMIDE	228	78
			N-ETHYLIMIDE	166-67	23
III	3,4-METHYLENE-DIOXYPHTHALIC ACID $C_9H_6O_6$	NONE REPORTED			
			N-ETHYLIMIDE	124-25	23,80
IV	HEMIPINIC ACID $C_{10}H_{10}O_6$	173-75 177			81 79
			ANHYDRIDE	166-67	79
			IMIDE	230	79
			DIMETHYLESTER	61-62	79
			N-METHYLIMIDE	166-67	79

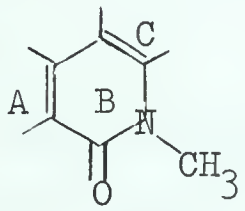
NO.		M.p. °C.	DERIVATIVE	M.p. °C.	REFERENCE
V	m-HEMIPINIC ACID $C_{10}H_{10}O_6$	193-99			81
			ANHYDRIDE	175	79
			N-ETHYLIMIDE	229-30	77,79
			N-METHYLIMIDE	254-56	35,81



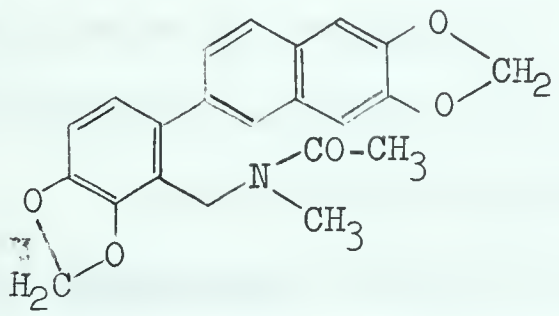
VI 9,10-DIHYDRO-



VII 9-OXY-



VIII N-ACETYLANHYDRO-CHELIDONINE
$C_{22}H_{19}O_5N$ — 20,57



It will be seen that this group which may be classified as isoquinoline alkaloids (2,33,82) may be further divided into two subgroups; those with the fully aromatic structure, the quaternary bases sanguinarine, chelerythrine, xanthofagarine and avicine, and those in which rings B and C are fully reduced, the tertiary bases chelidonine, methoxychelidonine and homochelidonine. A series of syntheses by T. Richardson (67) et al (41,68,69) confirmed the structures for chelidonine, chelerythrine, and sanguinarine and laid the base work for the syntheses carried out by K.W. Gopinath et al (49) and Arthur (50) to confirm the structures of xanthofagarine and avicine. The latter subgroup has not been reported in bloodroot. It is interesting to point out that all of these alkaloids, so far characterized, have a methlenedioxy group in the D ring invariably in the 2',3'-position. (See I, TABLE III)

By appropriate reactions these alkaloids can form the stable 9-oxy- and the 9,10-dihydro-derivative (26,35,37,45). (See VI and VII, TABLE III)

It has recently been stated (38) that these alkaloids react with nucleophilic reagents at position 9 to form the 9-substituted hydro derivative. This was recognized early in the present investigation by means of nuclear magnetic resonance (NMR) and infrared (IR) spectra and will be discussed more extensively in the experimental section. This reaction with the nucleophilic reagent (solvent) would help to explain the variations in melting points reported in the literature.

Although these alkaloids were thought to occur only in the family Papaveraceae, during the last eleven years they have been isolated from members of the family Rutaceae. (35,36,37,84,86)

A. CHELIDONINE SUBGROUP

1. CHELIDONINE

Chelidonine (V, TABLE I) was origionally extracted from Chelidonium majus (52,58,87) but has since been found in Bacconia frutescens (43), Stylophorum diphyllum (88), Dicranostigma franchetionum (89) and Glaucium corniculus (53).

It is a white, crystalline, tertiary base and forms colorless salts. The base is readily soluble in ethanol and ether but insoluble in water (39). Chelidonine has an optical rotation of +115.40 in ethanol and +117.4 in chloroform (26).

The structure of chelidonine was established by von Bruchhausen and Bersch (21,22) and later confirmed by Späth and Kuffner (23,90). Chelidonine forms an optically active O-acetylderivative, whose melting point varies from reference to reference, seemingly due to the solvent of crystallization (20,38,52,57,91). At the boiling point of acetic anhydride ring scission and dehydration occur to give the optically inactive N-acetylanhydrochelidonine, $C_{22}H_{19}O_5N$, m.p. 152-53°(20,57), (VIII, TABLE III). Oxidation of O-acetylchelidonine with mercuric acetate (57) yields a compound which on contact with acid loses water and acetic acid to form dihydrosanguinarine, which in turn is oxidized by air to sanguinarine (39).

Permanganate oxidation of chelidonine yields hydrastic and 3,4-methylene-dioxyphthalic acids (II and III, TABLE III) (21,23,80). These degradation fragments indicate that the methylenedioxy groups are not in the same relative positions on the two nuclei. This series of degradations established the partial structure (Figure I) for chelidonine. The hydroxyl group was placed at C_{10} on biogenetic grounds (21,22) and because degradation products demonstrated it was not in rings A, B or D.

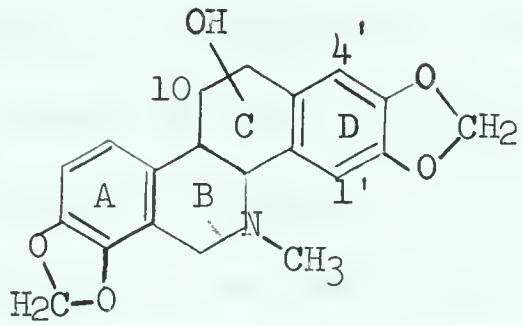


Figure I

In 1958 Bersch (92) used infrared (IR) spectroscopy to show that the hydroxyl group must be at C_{10}, and this has been subsequently proven by Palmer and Martin (93) who used chemical and nuclear magnetic resonanance (NMR) techniques.

2. HOMOCHELIDONINE

The structure for homochelidonine (VII, TABLE I) was proven similarly to that of chelidonine, only instead of obtaining sanguinarine, chelerythrine was the final product (39,44), thus proving that a methylene-dioxy group was replaced by two methoxyls in homochelidonine.

This alkaloid, which is known to occur only in Ch. majus (58), is colorless and forms amorphous salts, with the exception of the aurichloride which forms reddish-yellow needles from alcohol (39).

3. METHOXYCHELIDONINE

Gadamer and Winterfeld (20) are the only persons ever to obtain this alkaloid. They extracted it from Ch. majus and crystallized it from alcohol as prisims, m.p. 221°, $[\alpha]_D$ +115.8°. It forms a crystalline hydrochloride, aurichloride, and an O-acetylchloroaurate although the O-acetyl derivative is amorphous.

Although there are four unsubstituted positions in the two benzene nuclei, only two (1' and 6, Figure I) are reasonable for the sight of the methoxyl on biogenetic grounds. In either case, the biogenetic precursor would be 3,4,5-trialkoxy derivatives; but a satisfactory choice between the two positions is not possible. However, von Bruchhausen and Bersch (21), accepting Gadamer and Winterfeld's view that this base is methoxychelidonine, prefer position 1' (26,39). If the methoxyl group does exist, we would agree with Arthur (35) rather than with von Bruchhaussen and Bersch to its positioning. However, work in this laboratory would tend to indicate that this alkaloid is an alcoholate, probably at position 9, rather than a naturally occurring alkaloid. Circumstantial support for our proposal is that as recently as 1954 Salvik (94), on a thorough investigation of Ch. majus, found no methoxychelidonine although he did find several new alkaloids.

C. SANGUINARINE SUBGROUP

1. CHELERYTHRINE

Chelerythrine (II, TABLE I) is of widespread occurrence in Papaveraceae and has recently been isolated as a minor constituent of several rutaceous plants. This alkaloid was first reported by Probst (24) in 1839 from Ch. majus. Subsequently it has been isolated from Ch.majus by others (25,52,58,94) and also from Sanguinaria canadensis (25,27,28,29,95,96), Bocconia (Macleaya) arborea (97), B.fructescens (43), B. cordata (61,98,99), B.pearcei (100,101,102), Macleaya microcarpa (103), Eschscholtzia californica (96,101,102), Glaucium fimbrilligerum (104), G. flavum (96,105), G. corniculus (53), G. vitellinum (106), G. elegans (107), Dicranostigma lactucoides (102), D. franchetianum (108), Argemone mexicana (109), A. alba (110), Dicentia spectabilis (111), Hypeconum trilobum (112),

H. procumbens (113), H. leptacarpum (113), and Stylophorum diphyllum (114). From the Rutaceae, it has been isolated from Fagara semiarticulata (45), Xanthoxylum rhetsa (86), X. brachyacanthum (84), X. venificium (84) and Toddalia acculeata (85).

Chelerythrine itself is colorless and crystallizes from alcohol as an optically inactive prismatic leaflet, m.p. 207°, containing one molecule of alcohol (25). When sufficiently pure it melts at 210° (97). Its salts, which are quaternary, are generally an intense yellow. Even when allowed to stand in the air this alkaloid will absorb carbon dioxide rapidly and turn yellow (39).

Chelerythrine is freely soluble in chloroform; slightly soluble in alcohol and ether is insoluble in water. The salts have poor solubility in water (60). The alkaloid, when on paper, fluoresces blue (27); while the solid has a yellow fluorescence (94).

Govindachari (85) reported that the preparation of the 9-oxy-derivative with alkaline ferricyanide proceeds with relative ease, while Scheuer (45) claimed that the reaction proceeds only at higher temperatures. Dihydrochelerythrine has been reported to occur naturally (45,109,115).

With Grignard reagents, α-alkyldihydrochelerythrines are formed analogous with the alkyldihydroberberines (39) (see "Berberine" page 22) and it reacts with phenylhydrazine as if it contains a carbonyl group (47). Gadamer (44) showed that these reactions, which appear to indicate a carbonyl group, occur in the same way as with berberine. Two of the oxygen atoms were shown to be present as a methylenedioxy group, and the other two as methoxyls. On these grounds Gadamer regarded chelerythrine as a quaternary base, which on liberation from its salts passed into the carbinol form. In subsequent papers Gadamer suggested that the contradictory observations recorded regarding chelerythrine and sanguinarine were in part due

to the difficulty in separating these alkaloids (39). Späth and Kuffner (23,42,90) found that chelerythrine chloride on gentle oxidation by potassium permanganate yields the methylimide of 3,4-dimethoxyphthalic acid, and on more energetic oxidation, 4,5-methylenedioxyphthalic acid (hydrastic acid) was also produced. On distillation with zinc dust they found that chelerythrine chloride furnished 1,2-benzophenanthridine, $C_{17}H_{11}N$ (39,65) m.p. 136° (65,66). This latter compound forms a hydrochloride, m.p. 235°, and a picrate, m.p. 256° (65). For further information on this compound and its derivatives the reader is referred to the literature cited (35,37,41,49, 50,67-76).

Further observations (42) showed that dihydrochelerythrine on treatment with phloroglucinol and sulfuric acid and subsequent methylation of the dihydric phenolic base so formed, yields tetramethoxy-N-methyl-dihydro-1,2-benzophenanthridine, m.p. 182-3° (35,45,69). This compound is also obtainable from sanguinarine (39,42). These reactions led to the correct structure which has been subsequently proven by synthesis (41).

2. SANGUINARINE

Like chelerythrine, this alkaloid is widespread in the Papaveraceae; but unlike chelerythrine, it has not as yet been reported in Rutaceae.

In 1829 Dana (19) isolated sanguinarine (I, TABLE I) from S. canadensis, and thus became the first to isolate a 1,2-benzophenanthridine alkaloid. This sample was found to be impure, and the alkaloid was not obtained in pure condition until 1924, when it was obtained under the name ψ-chelerythrine from chelidonine (20).

This alkaloid is reported to occur in S. canadensis (25,27,28,29, 95,96), Glaucium fimbrilligerum (104), G. flavum (105), G. corniculus (53),

G. vitellinum (106), G. elegans (107), Bacconia cordata (1,98), B. pearcei (102), B. microcarpa (103), Stylophorum diphyllum (88), Dicranostigma franchetianum (89), D. lactucoides (102), Argemone mexicana (109,116), A. alba (110), Eschscholtzia californica (101), Dicentia spectabilis (111), Hypeconum trilobum (112), H. procumbens (113), H. leptocarpum (113), Meconopsis aculeata (117), M. rudis (117), M. horridula (117), M. latifolia (117), M. betonicifolica (117), M. pancculata (117), and Corydalis incisa (118).

The natural alkaloid, which is very difficult to separate from chelerythrine and protopine (27), is colorless and forms needles from ethyl-acetate or ethanol, m.p. 213° (25,27); however, by removal of associated chelerythrine as the pseudocyanide and fractionation of the residue as the acid d-tartrate, Gadamer and Stichel (40) obtained sanguinarine, which crystallized from ether and melted at 242-3° after being heated at 225-30° for five minutes, or at 266° (28,38,40) when heated more rapidly. Crystallized from alcohol, it melts at 195-97° and is then probably an alcoholate (20). It is soluble in most organic solvents, with a bluish-violet fluorescence (39). It has a blue-violet fluorescence on paper (27) but the solid fluoresces orange (94).

With the exception of the colorless pseudocyanide, the salts are deep red (25).

Sanguinarine reacts with various nucleophilic reagents to form 9-hydrosanguinarine derivatives; thus supporting the quaternary ammonium structure. Two such derivatives are 9-benzyloxyhydrosanguinarine, m.p. 191° and 9-anilinosanguinarine, m.p. 239-40° (38).

Oxysanguinarine has been reported to be easily obtained from sanguinarine by warming it in a solution of concentrated sodium hydroxide (20). It has also been reported to occur naturally (28,119). Dihydrosanguinarine,

m.p. 188-89°, which occurs naturally (66,109,118), is formed similarly to dihydrochelerythrine (42).

Similarly to the way homochelidonine was converted to chelerythrine, chelidonine has been converted to sanguinarine (38,40).

The relationship between sanguinarine and chelerythrine was established by Späth and Kuffner (42), who showed that dihydrochelerythrine and dihydrosanguinarine, prepared from the natural alkaloids, on replacement of the methlenedioxy groups by methoxyl groups yielded the same substance, namely tetramethoxy-N-methyldihydro-1,2-benzophenanthridine, m.p. 182-83°.

3. XANTHOFAGARINE (NITIDINE)

In 1959 this alkaloid was isolated from the root bark and wood of Zanthoxylum nitidum by Arthur (35), who called it nitidine, and it was shown to be a benzophenanthridine alkaloid with a new substitution pattern (III, TABLE I). McDonald (36) showed that the previously known alkaloid, xanthofagarine, from Fagara macrophylla (48) was identical with nitidine, and in lieu of the fact that xanthofagarine was the first isolated, although not characterized, he suggested that this alkaloid be known as xanthofagarine.

The alkaloid, m.p. 278-82°, is colorless, although on exposure to air it becomes a greenish-yellow (36).

The salts are yellow and decompose with heat to give nor-xanthofagarine.

On paper the alkaloid fluoresces red-brown with a green phosphorescence, which is due to oxyxanthofagarine. Oxyxanthofagarine itself has a blue fluorescence (36).

Although the base is relatively insoluble in all solvents, prolonged boiling in excess solvent effects solution in glacial acetic acid, methanol, ethanol and water. It is insoluble in chloroform, acetone, ether and hydrocarbons (36). The salts, however, are quite soluble in cold water (35).

Oxyxanthofagarine is reported to occur naturally, and is also easily obtained, as well as some dihydroxanthofagarine, by basification of an aqueous solution of xanthofagarine. This is suggested to be due to disproportionation (35). Since dihydroxanthofagarine was easily oxidized by air (35,36) it was separated from oxyxanthofagarine by chromatography under argon (35). Oxyxanthofagarine does not form salts and is insoluble in water (35).

Dihydroxanthofagarine, crystallizes from ethanol as colorless, elongated prisms which rapidly turn yellow on exposure to air. Dihydroxanthofagarine hydrochloride, m.p. 215-16°, crystallizes from chloroform/ethanol as does the white hydrogen sulfate derivative, m.p. 290-92°. The methiodide is hard to obtain, but crystallizes from methanol as orange needles, m.p. 268° (35).

Oxyxanthofagarine was shown to be isomeric with, but not identical to oxychelerythrine. Furthermore, the substitution pattern was shown to be different by converting both to the tetramethoxy derivatives.

Degradative oxidation of oxyxanthofagarine, which was difficult to control, gave N-methyl-m-hemipinimide. This suggested that the methoxyl groups were substituted in the 6 and 7 positions of the benzophenanthridine skeleton, and not in the 7 and 8 positions as in all previously isolated benzophenanthridine alkaloids. This was subsequently proven by synthesis (35,49,50).

4. AVICINE

This alkaloid (IV, TABLE I) was isolated from the rootbark of Zanthoxylum avicinnae, and is related to xanthofagarine as sanguinarine is related to chelerythrine (37).

Here again, when an aqueous solution was basified, the alkaloid yielded a mixture of oxyavicine and dihydroavicine, although less readily than with xanthofagarine. Oxyavicine does not form salts, although it can be reduced to dihydroavicine. Avicine is colorless as the base, but forms yellow salts.

The substitution pattern of avicine was proven to be the same as for xanthofagarine by formation of the same tetramethoxy compound from both (37).

The reactions of the fully unsaturated alkaloids are interesting. Although the bases are colorless or faintly colored, their salts are brightly colored, and when subjected to pyrolysis these salts decompose to give their respective nor-compound (28,36,38,41,45,49). The bases form the high melting oxy-compounds on oxidation with alkaline potassium ferricyanide (45,85). With aqueous potassium cyanide they give the sparingly soluble, colorless pseudocyanides, and appear to be the derivative of choice. With zinc and hydrochloric acid they are reduced to the dihydro derivatives which have been reputed as being unstable compounds which revert to the free base on exposure to air (35-37). A very wide range of melting points are reported for the bases depending on the solvent used for crystallization.

D. SUSPECTED BENZOPHENANTHRIDINES

From the reactions and behavior of some minor bases, they also would appear to be benzophenanthridine alkaloids (28,94,95). This, however, has not yet been proven.

1. CHELIRUBINE

This alkaloid has been detected in Ch. majus (94,120), Eschscholtzia

californica (101), Glaucium flavum (55,105), G. corniculus (53), G. vitellinum (106), G. elegans (107), Macleaya microcarpa (103), Dicranostigma franchetianum (108), D. lactucoides (121), Stylophorum diphyllum (114), Hypeconum procumbens (113), H. leptocarpum (113), Papaver rhoeas (111), Dicentia spectabilis (111) and S. canadensis (28). However, it has been isolated from only a few of these plants, and then only in small amounts. (28,94,101,106,114,120)

It is reported as melting at 257-58° (Et_2O), needles 217-18° rapid heat, 230-31° (EtOH); while its hydrochloride melts at 282-83° (28). Its pseudocyanide melts at 269-70° (101), 273-74° (EtOH) (28). The alkaloid has a red-violet fluorescence on paper and forms purple salts (28).

2. CHELILUTINE

This alkaloid has been detected in Eschscholtzia californica (101), Macleaya microcarpa (103), Papaver rhoeas (111), Ch. majus (94) and S. canadensis (28), but isolated only from the latter two.

The alkaloid has a salmon-colored fluorescence on paper (94), melts at 229-30° (Et_2O) or 202-03° (EtOH) and forms orange salts. The hydrochloride melts at 197-8° (H_2O) and the pseudocyanide at 270-71° (EtOH) (28).

3. SANGUIRUBINE

This alkaloid, known to occur only in S. canadensis is optically inactive and melts at 252-53° (Et_2O) or 224-25° (EtOH). It forms a hydrochloride which melts at 237-38° (EtOH) (28).

4. SANGUILUTINE

This alkaloid also is known only to occur in S. canadensis. It is also optically inactive and melts at 246-47° (Et_2O) or 211-12° (EtOH).

Its hydrochloride melts at 163-4° (EtOH) and its pseudocyanide at 232-3° (EtOH) (28).

E. ASSOCIATED ALKALOIDS

As well as the above benzophenanthridine alkaloids, other structurally related alkaloids of widespread occurrence have been reported to occur in plants containing benzophenanthridine alkaloids. Among the more important are protopine (30) (fumarine, macleyine), allocryptopine (30) (β-homochelidonine, α-fagarine) and berberine (63) (chelidoxanthin). Minor alkaloids of interest include coptisine [bis (methylenedioxy) protoberberine] (28,63,108,109), stylopine (tetrahydrocoptisine) (63,89,120,122), canadine (tetrahydroberberine) (63,84), corydine and isocorydine (53,55,123), glaucine (123), cryptopine (30,103), and cryptocavine (30).

1. ALLOCRYPTOPINE

Allocryptopine (II, TABLE II) is of very widespread occurrence in the Papaveraceae (30) and has also been found in the Rutaceae (30,124).

The alkaloid occurs in two allotropic forms; the α-form, m.p. 160°, and the β-form, m.p. 170° (61). The older names of β- and γ-homochelidonine were based upon an assumed relationship to chelidonine and are no longer acceptable (30).

Allocryptopine, which is sparingly soluble in methanol but readily soluble in chloroform, forms colorless salts, of which the aurichloride melts at 187° (52).

Of its many reactions, one of great biogenetic importance is that it can be converted to dihydroberberine methochloride by the action of phosphorous oxychloride (30).

2. PROTOPINE

Protopine (I, TABLE II) is one of the most widespread of all alkaloids, having been isolated from a long list of plants (30,34, 89,102,103,107,117), chiefly from Papaveraceae and Fumariaceae.

The alkaloid is a colorless, crystalline solid, m.p. 201° (59), 207° (34), soluble in chloroform, slightly soluble in ethanol, ether, ethyl acetate, benzene, and petroleum ether, but insoluble in water (34). It forms a yellow aurichloride, a colorless benzoate (60), and a picrate (20).

3. BERBERINE

Berberine (IV, TABLE II) occurs widely among the Papaveraceae and is also found in the Rutaceae (63). It crystallizes from water or dilute ethanol as brilliant yellow needles containing 5.5 moles of water. When dried at 100° it loses three moles of water and becomes yellowish-brown and finally decomposes at 160° (63,64). When recrystallized from ether, the yellow needles melt at 145° (60). One gram is soluble in 4.5 ml. of water at 21° and in 100 ml. of ethanol. It is only slightly soluble in chloroform and benzene (63,64).

Berberine forms salts, with the loss of one molecule of water, which are mostly a dull yellow color. These salts are poorly soluble in water and hence crystallize readily (125).

A study of the work which resulted in the correct constitutional formula for berberine can begin with the researches of Perkin (125,126) dealing with the oxidation of the alkaloid by permanganate. He found that excess permanganate yielded mainly hemipinic acid (IV, TABLE III); whereas, the oxidation of berberine by amounts of permanganate insufficient to effect oxidation to hemipinic acid gave five products: oxyberberine, $C_{20}H_{17}O_5N$; dioxyberberine, $C_{20}H_{17}O_6N$; berberal, $C_{20}H_{17}O_7N$; anhydroberberilic acid, $C_{20}H_{17}O_8N$; and berberilic acid, $C_{20}H_{19}O_9N$ (63,125,126).

The necessity for at least two formulae to represent berberine was shown by Gadamer (12) who observed that on adding barium hydroxide to a berberine sulfate solution, a brownish-red, strongly alkaline solution of the free base is obtained, which with excess of sodium hydroxide yields berberinal (supposed aldehyde form of berberine) (64). This yields an oxime, m.p. 165°, and on treatment with concentrate sodium hydroxide yields oxyberberine and dihydroberberine, thus behaving like an aromatic aldehyde. Tinkler (128) has observed that berberine and its salts show the same ultra-violet absorption spectra, while Gadamer's berberinal shows an absorption spectrum almost identical with that of Freund and Beck's α-methyldihydroberberine (129), which would appear to be a derivative of a carbinol form of berberine. From this and other data it has been deduced that berberine is represented as the carbinol form and can be converted to oxyberberine and dihydroberberine by the action of strong alkali (64).

F. BIOSYNTHESIS

The processes by which alkaloids are synthesized in plants have been the subjects of study and speculation among organic chemists and biochemists. The structural types found in different classes of alkaloids are so diverse that it is impossible to develop a single biogenetic hypothesis to include all alkaloids.

In fact, for this group of alkaloids we have several hypotheses of which only one (130) stands up to tracer experiments and incorporates related alkaloids.

A larger number of alkaloids may be hypothetically derived from norlaudanosoline (I, Figure II) which is plausibly formed from 3,4-dihydroxy-phenylalanine and 3,4-dihydroxyphenylacetaldehyde (130). Oxidation and

methylation of norlaudanosoline yields papaverine (II), (131). Oxidative coupling of the two phenolic rings of norlaudanosoline yields the aporphine alkaloids, which can have two possible hydroxylation patterns, represented by the alkaloids corydine (III) and dicentrine (IV) (130). An alternative mode of oxidative coupling via the intermediates V, VI and VII yields the alkaloids sinomenine (VIII), thebaine (IX), morphine (X) (130).

Reaction of norlaudanosoline with formaldehyde, or its biological equivalent, in a Mannich reaction leads to the isomers XI and XII (Figure III) (130), which may be regarded as precursors of the alkaloids canadine (XIII) and coreximine (XIV) respectively.

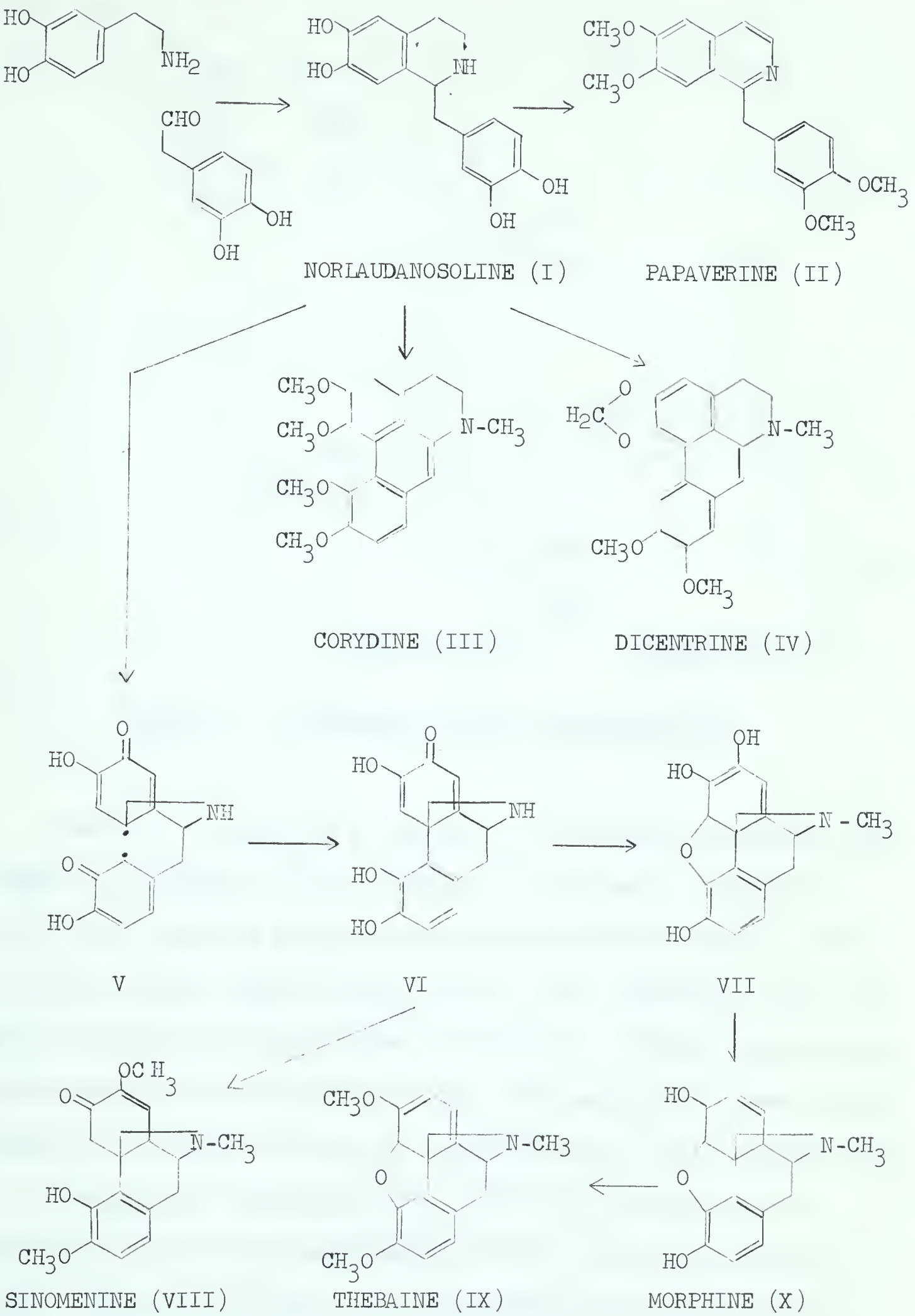
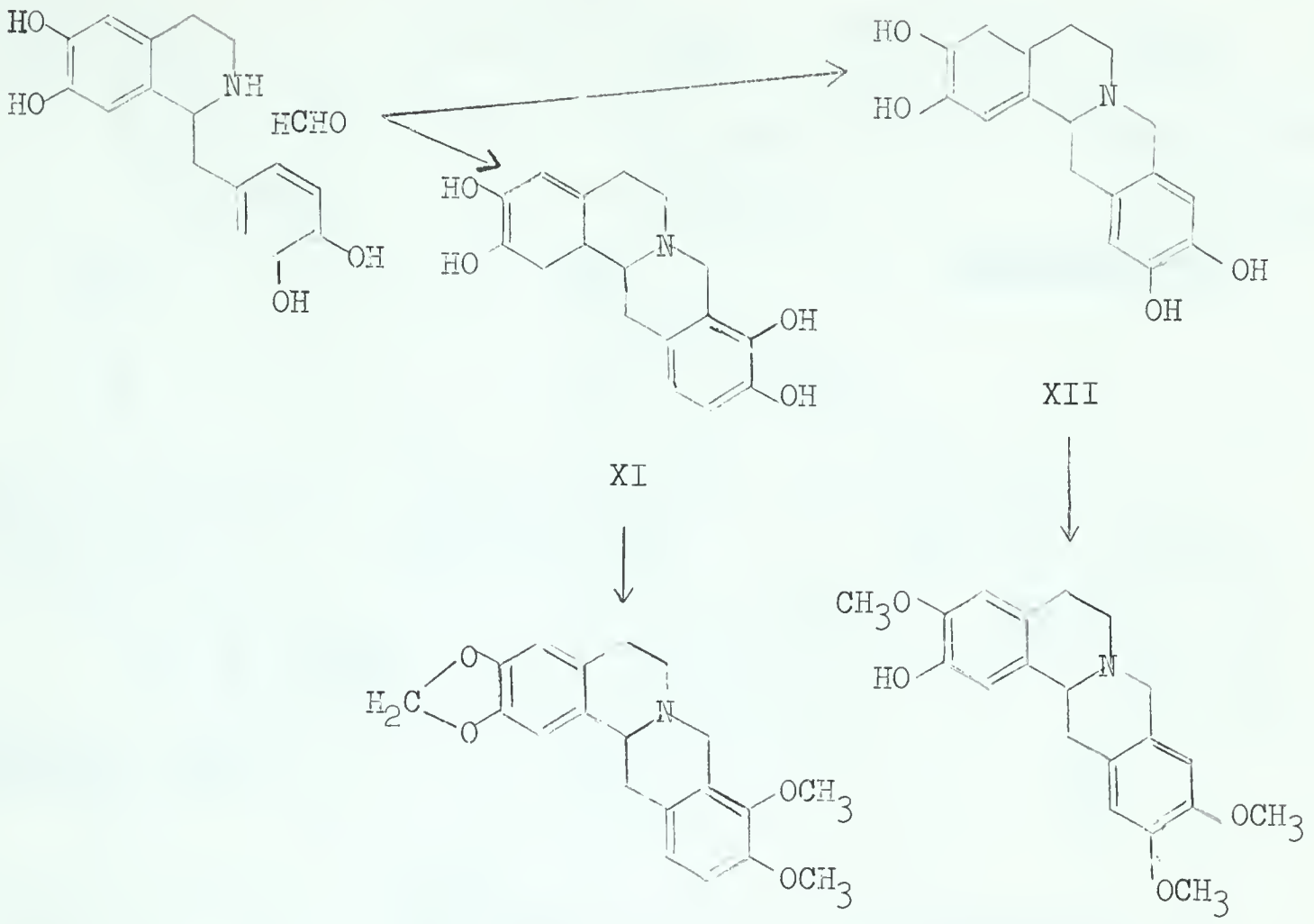


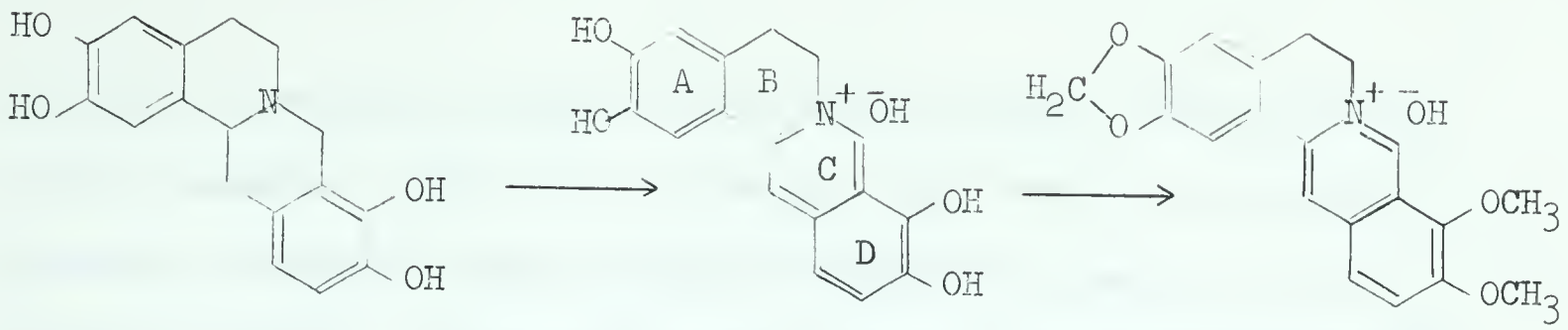
FIGURE II. SOME ALKALOIDS HYPOTHETICALLY DERIVED FROM NORLAUDANOSOLINE.

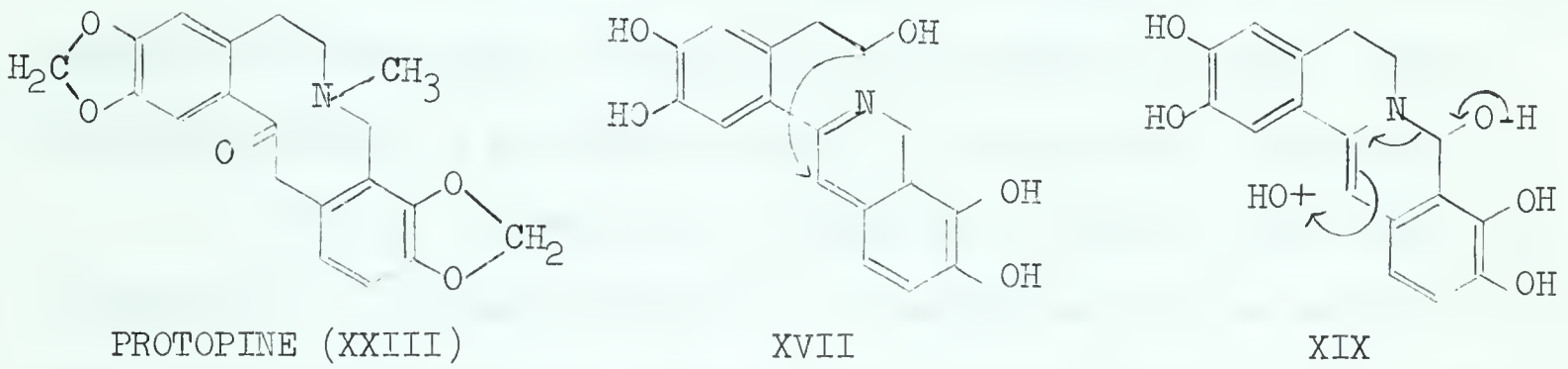


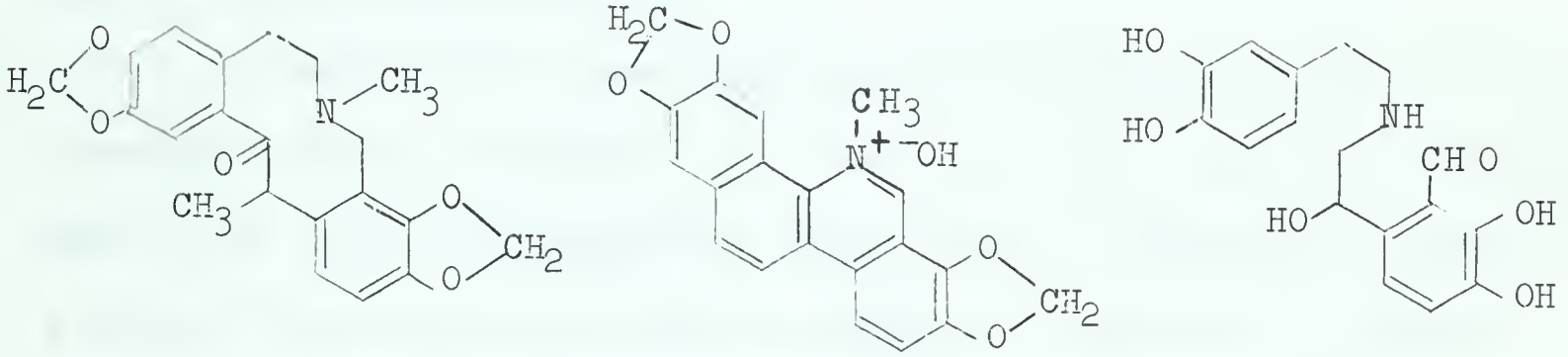
CANADINE (XIII) COREXIMINE (XIV)

FIGURE III. BIOGENESIS OF THE PROTOBERBERINES.

However, the majority of alkaloids of this type are derived from the isomer XI. Oxidation of this isomer, as illustrated in FIGURE IV, (130,132) yields compound XV which on alkylation affords berberine (XVI) (130,133,134). Tracer work is consistent with this biogenetic scheme for berberine (130,135,136,152 and references therein). Further hypothetical transformations of the intermediate XV are also represented schematically in FIGURE IV. Fission of this ring B could plausibly yield compound XVII and recyclization as illustrated could yield the ring system of the benzophenanthridines of the sanguinarine (XVIII) substitution pattern (137,130). Tyrosine-2-C^{14} has been incorporated into chelidonine and sanguinarine in accordance with this scheme (80,130). Fission of ring C







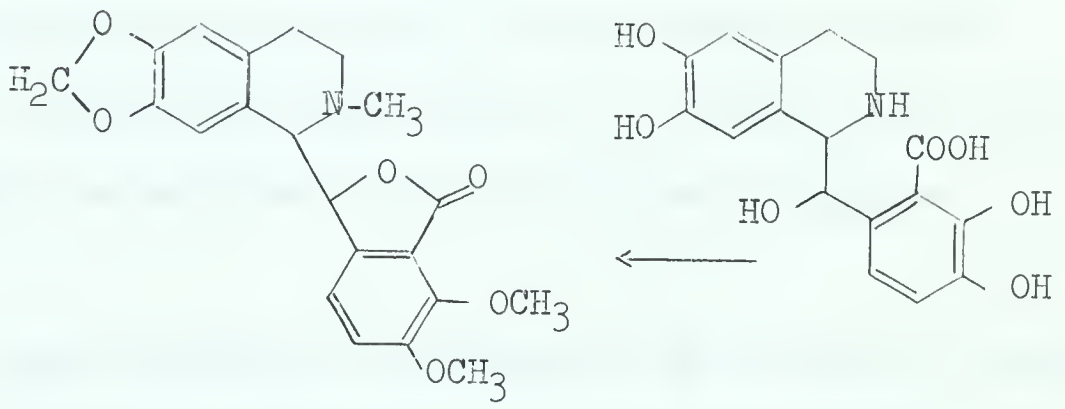


FIGURE IV. HYPOTHETICAL BIOGENESIS OF SOME ALKALOIDS RELATED TO BERBERINE.

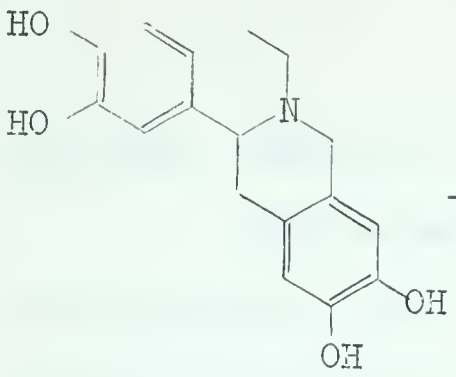
via the intermediate XIX leads to the hydroxyaldehyde (XX) which upon further oxidation leads to the hydroxyacid (XXI) which is considered a plausible precursor of many phthalideisoquinoline alkaloids such as hydrastine (XXII). Alternative schemes for this class of alkaloid have been suggested by Wenkert (138) and Manske (139).

Oxidative cleavage of a C-N bond in the protoberberine XI affords another structural type, represented by the alkaloid protopine (XXIII). Corycavine (XXIV) is presumably formed by C-methylation of protopine.

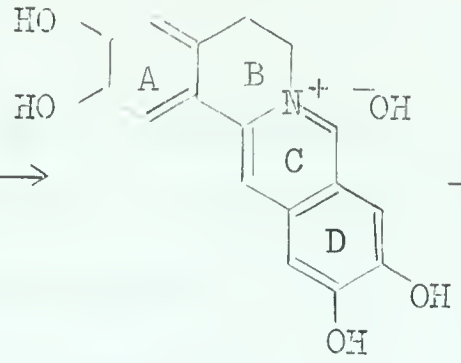
Although the majority of alkaloids are derived from isomer (XI) (Figure III) by logical analogy with the aporphines and the protoberberines, there is no reason why the reactions that occur in figure IV could also not occur starting from isomer XII.

Starting with isomer XII and passing through intermediate XXV coreximine (XXVI) is obtained (140). Fission of ring C via the intermediate XXVII leads to the hydroxyaldehyde XXVIII which is a plausible precursor to a nucleus for phthalideisoquinoline alkaloids, represented by compound XXIX, of yet unknown substitution (141). Similarly, fission of ring B could yield compound XXX which on recyclization as illustrated would give rise to the benzophenanthridine alkaloids of the xanthofagarine (XXXI) substitution pattern. We could also expect to find, in nature, the unknown alkaloids (XXXII) related to chelidonine but with the xanthofagarine substitution pattern.

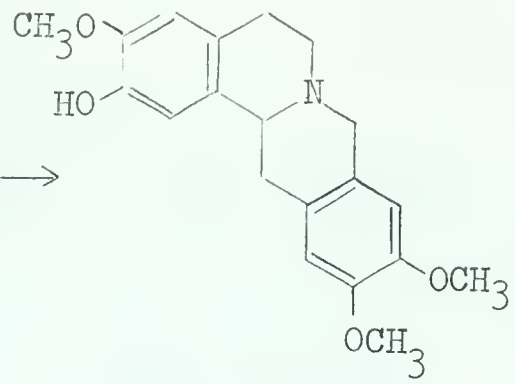
Yet another type of alkaloid is afforded by the oxidative cleavage of a C-N bond in XII. This type of reaction would give rise to fagarine II (XXXIII), (also III, TABLE II). It is interesting to note that fagarine III (82), of undetermined structure, could quite easily be a tetramethoxy-protopine because it has an ultra-violet spectra closely resembling the other



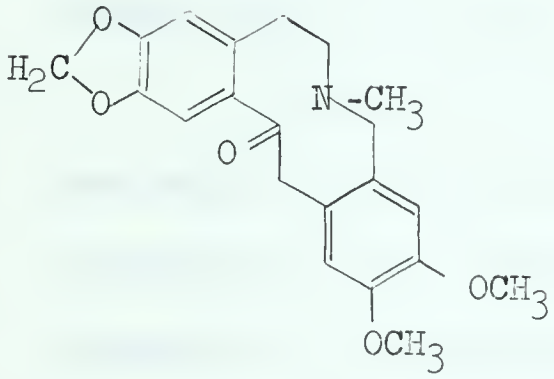
XII



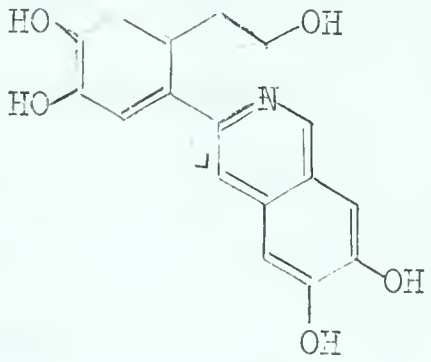
XXV



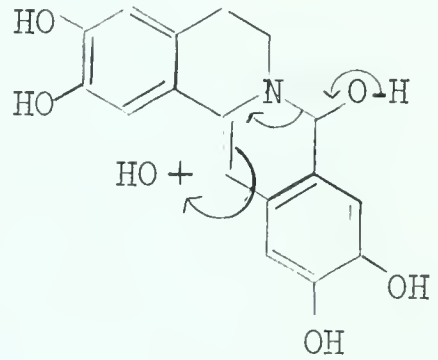
COREXIMINE (XXVI)



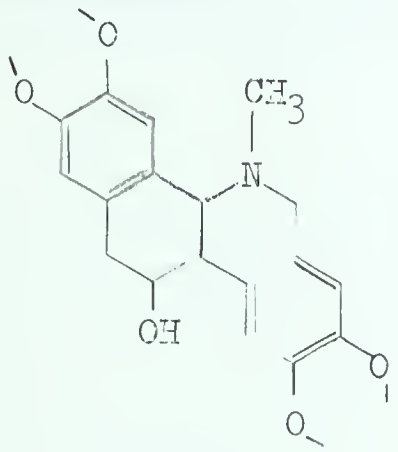
FAGARINE II (XXXIII)



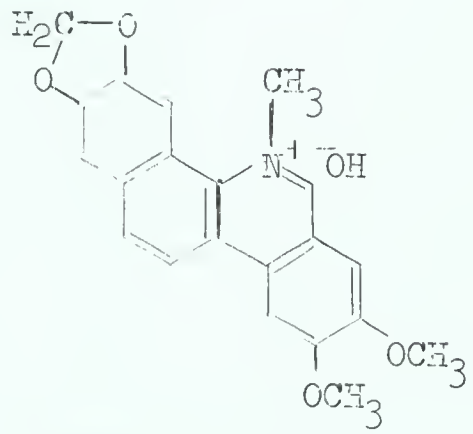
XXX



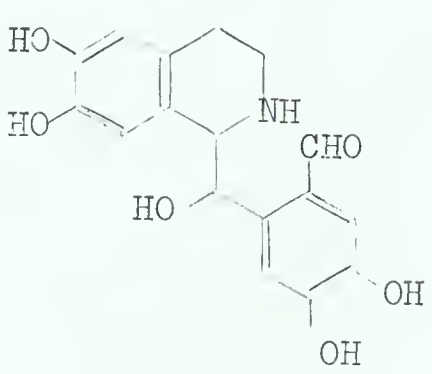
XXVII



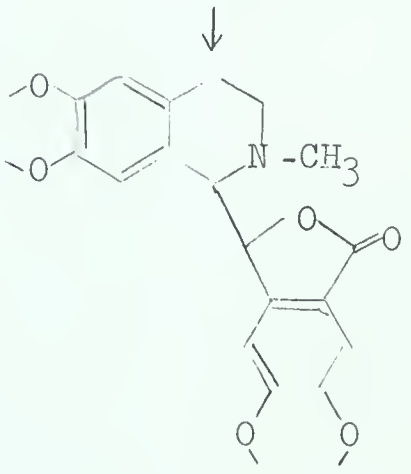
XXXII



XANTHOFAGARINE (XXXI)



XXVIII



XXIX

FIGURE V. HYPOTHETICAL BIOGENESIS OF SOME ALKALOIDS RELATED TO COREXIMINE.

protopine alkaloids but has no methylenedioxy group (82).

Such processes as C-, O- and N-methylation, O, O-methylenation, O, N-acylation, etc., are considered to occur late in the biogenesis and to occur independently of the formation of the nucleus. These one carbon fragments are considered to originate from formic-acid, a carbinolamine or from amino acids like glycine, choline or methionine (47,132,134). Tracer experiments are confirming these hypothesis (16,132).

Not only do the tracer experiments tend to indicate a common precursor for berberine and the benzophenanthridine alkaloids, but the coexistence of the berberine alkaloids and the 1,2-benzophenanthridine alkaloids in the Papaveraceae [Ch. majus, (32), Glaucium corniculatum (53) and several other species (28,103,108,109,121)] and Rutaceae [Toddalia aculeata (85)] is strong circumstantial evidence in favor of their biosynthesis from common precursors (80).

PART III

DISCUSSION OF EXPERIMENTAL WORK

Although the alkaloids sanguinarine and chelerythrine were known earlier (19,24) it was not until 1891 that they, together with protopine and γ-homochelidonine (α-allocryptopine), were isolated from Sanguinaria canadensis (25) and it was not until 1930 (21) that the correct structures were elucidated. Furthermore, an investigation (28) as recently as 1960 revealed the presence of several alkaloids not previously detected in S. canadensis. It was thought that a comprehensive chemical investigation of the alkaloids of bloodroot was in order.

Therefore, the plan of this investigation was to isolate and characterize as many alkaloids from S. canadensis as possible, paying particular attention to the benzophenanthridine alkaloids.

In the light of previous work (25,27-29,95,96), it was expected that chiefly sanguinarine and chelerythrine with lesser amounts of protopine and α-allocryptopine would be obtained.

Chemical and physical separation and purification of these alkaloids, as others have found (27,58,65,87), proved difficult. The benzophenanthridine fraction was separated from the other alkaloids in a relatively pure state by means of an alumina (activity I) column. When separation of these alkaloids was not possible by column chromatography using alumina and two types of silicagel [silicagel for column chromatography and silicagel for thin layer chromatography (TLC)] separation was attempted using differences in solubilities. The only advantage this method held over the column was that it was possible to isolate some α-allocryptopine in a relatively pure state. Since the only good method of separation available appeared to be TLC, a total of 7.19 g. of the enriched benzophenanthridine fraction was applied to 240 thick plates. A total of 6.187 g. of separated alkaloids were recovered, 2142 mg. of sanguinarine, 382 mg. of base 2, 1827 mg. of

chelerythrine, 1565 mg. of base 4, 24 mg. of base 5 and 247 mg. of α-allocryptopine. These alkaloids, which were at least 90% pure, were recrystallized from 95% ethanol until they were homogenous by TLC.

Ultra-violet absorption spectra of bases 1 to 4 suggested that they were closely related in structure, but not related to the partially aromatic benzophenanthridine base chelidonine. Hence, since only two benzophenanthridine alkaloids (sanguinarine and chelerythrine) had been previously isolated from S. canadensis, bases 2 and 4 were chosen for further intensive study.

Bases 1 and 3 were readily identified as sanguinarine and chelerythrine, by means of comparative paper chromatography, NMR spectra, mixed melting points, and elemental analysis. Further proof that base 1 was sanguinarine was obtained by direct comparison (mixed melting point and infrared spectra) with an authentic sample of sanguinarine gratefully received from Dr. W.I. Taylor. (Ciba Pharmaceuticals, Summit, N.J.).

Bases 2 and 4, however, were found to be two entirely new benzophenanthridine alkaloids. NMR spectra showed that these alkaloids had three and five methoxyl groups respectively and base 2 had one methlenedioxy group. This was substantiated by Labat's test (positive for base 2, negative for base 4) and elemental analysis of the nor-derivatives [base 2: $C_{21}H_{17}O_5N$, $(OCH_3)_3$; base 4: $C_{22}H_{21}O_5N$, $(OCH_3)_5$]. Mass spectra, which are discussed later, were obtained for bases 1 and 4 and were found to have a similar fragmentation pattern. On the basis of the above data base 2 was named methoxychelerythrine and base 4 was called sanguinaricine and their proposed structures are shown in Figure XI.

Although these alkaloids were penta-substituted, the proposed substitution pattern had to be proven.

These four benzophenanthridine alkaloids, which are colorless

or buff colored but tend to become colored in presence of air, form intensely colored salts. Reproducible melting points of salts of these alkaloids, with the exception of the pseudocyanides, are almost impossible to obtain. This is due to the fact that these salts decompose prior to melting to give the nor-compound of which the melting point is recorded. If, however, the melting point is done rapidly a melting point, over a broad range, which is usually higher than the nor-compound, is obtained.

The salts of the benzophenanthridine alkaloids when sublimed "in vacuo" at 190° to 240° lose the N-methyl group to give the tertiary nor-compound. These nor-compounds give more reproducible melting points and therefore provide a more dependable means of identification than either the melting point of the free base or its salts. This loss of the N-methyl group was also a great aid in initially interpreting the NMR spectra and their integrations. Due to the greater stability of the nor-compounds they were sent for elemental analysis in preference to the initial free base or its salt.

Formation of the oxy-compounds, which could be readily identified by their infrared amide absorption maximum at 1650 $cm.^{-1}$, was found, eventually, to be a simple, rapid procedure. Oxidation procedures reported in the literature (35-37,45) were found to be unsatisfactory for at least one, and often all, of the alkaloids. One method, based on Arthur's disproportionation hypothesis (35), was attempted in which the quaternary salts are refluxed in a strong solution of potassium hydroxide. Only traces of the oxy-compounds were isolated from this reaction. A second reaction was done by suspending the alkaloid in aqueous potassium hydroxide and refluxing the mixture with potassium ferricyanide. This method gave yields of 60% and lower. Since solubility seemed to be the problem, other solvents

were sought which would completely dissolve the alkaloids in high concentrations and still be miscible with water. Since these alkaloids dissolve in glacial acetic acid, it was possible to use a modification of Leete's procedure (80). The alkaloid was dissolved in glacial acetic acid, water was added and the solution was neutralized with potassium hydroxide pellets. A solution of potassium hydroxide and potassium ferricyanide was then added and the mixture refluxed. This procedure gave lower yields than the above and hence was discarded. Dimethylsulfoxide (DMSO) proved to be the solvent of choice. The prodedure developed consisted of dissolving the alkaloid in DMSO, adding an equal volume of water and warming the solution on a water bath. To this was added a warm solution containing potassium hydroxide and potassium ferricyanide in equal amounts of DMSO and water. The combined solution was then refluxed for one hour, cooled and extracted with chloroform. After removal of starting material (denoted by lack of color in the aqueous phase) and some DMSO with 5% acetic acid the chloroform was washed with a saturated sodium bicarbonate solution and finally with water. The residue from the evaporation of the chloroform crystallized from 95% ethanol to give the oxy-compound. It was found to be important not to continue the reaction for more than one hour, as continuation beyond this time brought about decomposition of the oxy-compound to give the nor-compound. This latter reaction appeared to stop at near equilibrium between the two compounds.

An interesting and valuable observation was noted when the oxy-compounds, on silicagel, are sprayed with Dragendorff's alkaloidal reagent. In all cases they gave a purplish-blue spot. This coloration, which disappeared on heating, was also observed, although not so intense, when the plates were sprayed with ceric sulphate reagent.*

Contrary to general belief (35-37) the dihydro-compounds are

* See appendix.

quite stable, provided they are not allowed to come into contact with air in the presence of acid. The dihydro-derivatives of our four benzophenanthridine alkaloids, in crystalline form, have remained stable for a period of at least six months. They show little, if any tendency to revert to the base. Others (45,49,66,109,115,118) have also reported isolating dihydrochelerythrine, dihydrosanguinarine and dihydroxanthofagarine with no mention of lack of stability. With this in mind it is quite apparent that any isolation procedures which involve the use of an acid will fail.

However, when all solvents used in the crystallization contained a trace of ammonia, no difficulties were encountered. When sodium borohydride was added to a methanolic solution of the alkaloid the reaction proceeded extremely rapidly, completion being marked by a colorless solution. The crystalline dihydro-derivatives were collected in quantitative yields from "basic methanol".

The NMR spectra (TABLE VI and FIGURE XIV) proved to be the most valuable and useful physical data that were obtained. By a study of these spectra the substitution pattern and nature of the substituents can be quickly and facily established. The values of the various functions were found to be reproducible, varying only by a few cycles per second.

By a comparison of the spectra of sanguinarine and norsanguinarine it was possible to identify the N-methyl and methylenedioxy signals and also an O-ethyl group in sanguinarine crystallized from ethanol. That the O-ethyl group is present, but not as ethanol of crystallization was proved in two ways. First, there was no change in the NMR spectrum after shaking the alkaloidal solution in $CDCl_3$ with D_2O, which indicated that there was no hydroxyl group present. This suggested that the position of the O-ethyl group should be at 9 (see Figure VIII). A signal at 4.2-4.5τ, for a

benzylic proton, should also be present.

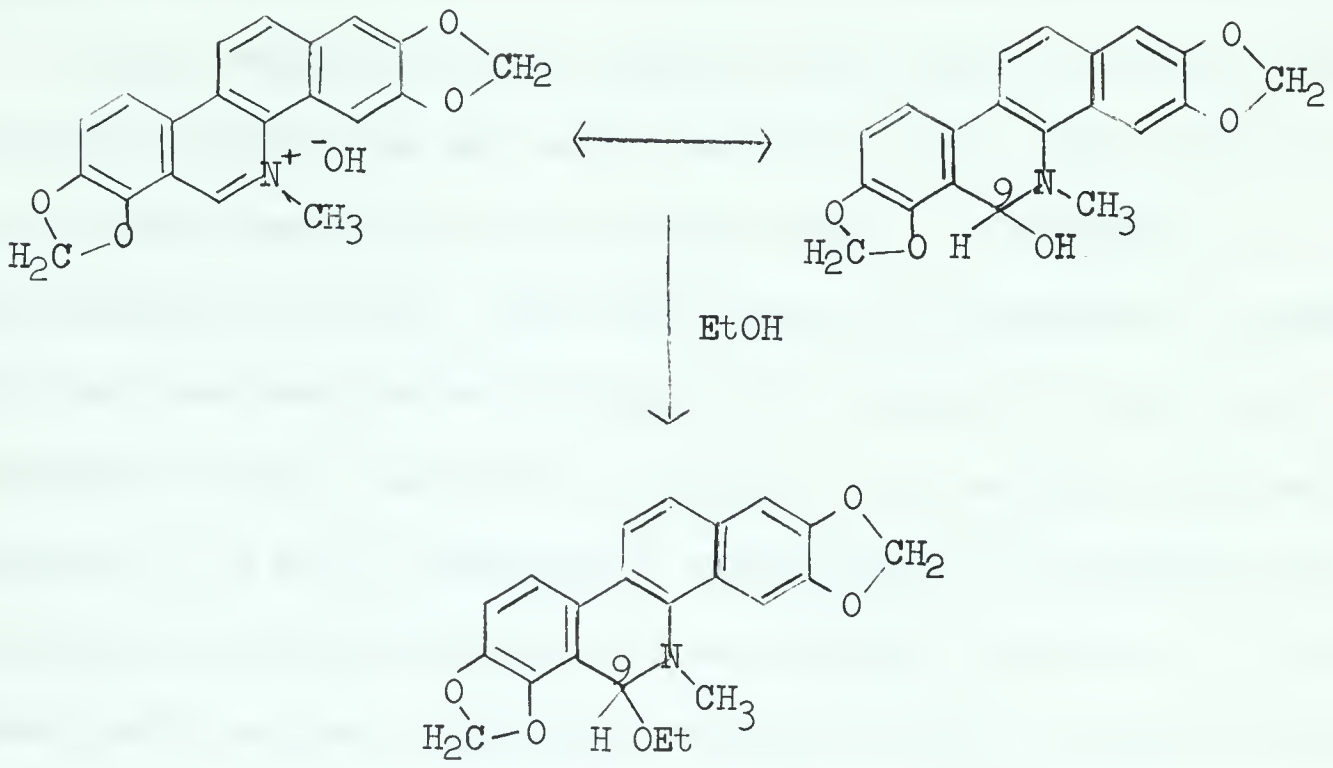


FIGURE VIII. RESONANCE HYBRIDS OF SANGUINARINE

Supporting this argument was the presence of such a signal for a benzylic proton at 4.34τ. In addition, there was no hydroxylic proton in the infrared spectrum. Further confirmation was obtained from the ultraviolet spectrum when a bathochromic shift was obtained on the addition of acid to the solution of the alkaloid. Such a shift can not be caused by a mere exchange of anions.

Consideration of the structures of sanguinarine and chelerythrine allowed, by comparison, the assignment of the signal at 3.96$\pm$.01τ to the methylenedioxy group of ring D (positions 2' and 3') and the signals at 6.05$\pm$.01τ and 6.08$\pm$.01τ to the methoxyl groups of ring A (positions 7 and 8). By comparison of the NMR spectrum of xanthofagarine (36) (spectrum obtained from a Varian A-60 instrument) with that of chelerythrine, it was shown that the signal at 6.08$\pm$.01τ was due to the position 7 methoxyl group,

while the signal at 6.05±.01τ was due to the position 8 methoxyl group and the signal at 6.18±.01τ was due to the methoxyl group at position 6.

Upon comparing the above spectra with those for bases 2 and 4 position 4 was chosen for the "extra" methoxyl group. This position is favored biogenetically since the hydroxyl group in chelidonine, a similar alkaloid, is on C_4. Also the choice of this position is suggested by the fact that there is no shifting of the signals for the alkoxy substituents in base 2 as would be expected if the methoxyl group was at position 4', 1' or 6. Furthermore, at position 4 the methoxyl would have essentially the same geometry as the methoxyl at position 7 (i.e. one adjacent proton and one substituted carbon) and hence this would account for the overlapping of the signals in the spectra. (At high resolution these tended to separate, but only by 1 or 2 cycles per second.)

Sanguinarine and chelerythrine have one more aromatic proton than do methoxychelerythrine and sanguinaricine and therefore must have a proton signal, probably a multiplet, that is absent in the spectra for the latter two. This signal was found and the proton was assigned to position 4 because a signal due to a proton at position 4 should be eliminated by the introduction of a methoxyl group at this position. This was shown by the lack of a multiplet at 2.35±.01τ -2.27±.01τ in the spectra for methoxy-chelerythrine and sanguinaricine. Further, the number of bands found for the protons at position 3 and 5 should be halved and shifted to higher field, due to the extra shielding of the methoxyl. Two such bands were found (although not well resolved). Thus the multiplets at 3.08±.04-2.95±.01τ and 2.19±.02τ in sanguinarine and chelerythrine decreased to a singlet at 3.37±.01τ and a doublet at 1.60±.01-1.46±.04τ respectively in the spectra for the pentasubstituted alkaloids. Since the proton at position 3 is obviously less hindered than the one at position 5, it was assigned to the

signal at 3.08±.04-2.95±.01τ in sanguinarine and chelerythrine and at 3.37 ± .01τ in the pentasubstituted alkaloids while the proton at position 5 was assigned to the signal at 2.19±.02τ in sanguinarine and chelerythrine and 1.60±.01-1.46±.04τ for methoxychelerythrine and sanguinaricine.

Of the three remaining protons one would expect doublets from the protons at positions 4' and 6 and a possible quartet (due to the N-methyl group) at position 1'. This would allow the assignment of the signal at 2.90±.01τ to position 4', 2.40±.1τ to position 6 and 2.57±.08τ to position 1'. A choice between position 4' and 6 was made on the basis that the signal for the proton at position 4' was constant until the ring D methylenedioxy was changed to two methoxyl groups whereas the signal for the proton at position 6 varied when the methylenedioxy was replaced by two methoxyl groups as well as when the "extra" methoxyl group was added. Although a choice was hard to make at that time, a spectrum of xanthofagarine or avicine should allow one to make a choice between them because they carry no proton at position 6. Although some of these assignments may not be correct, the assumptions made here are reinforced by the chemical data obtained.

The mass spectra of sanguinarine and sanguinaricine done primarily to determine molecular weights, showed very complex fragmentation patterns with numerous peaks below a mass/charge ratio of 100. The two spectra showed the same general fragmentation scheme, being almost identical below a mass/charge ratio of 228. Figures IX and X show the proposed hypothetical fragmentation patterns for these two alkaloids. The calculated ions being given with those found shown in brackets. The fragmentation pattern for sanguinaricine is given only to the point where it became identical with the spectrum for sanguinarine.

The infrared spectra (nujol) (TABLE VII and FIGURE XIII) of these alkaloids are very complex but show, when present, peaks for methylenedioxy (940, 1040, 1360 and 1480 $cm.^{-1}$) and methoxyl (shoulder in nujol or as a weak peak in halolube at 2850 $cm.^{-1}$). The nor- and dihydro- derivatives have spectral patterns almost identical to the base, however, the oxy-compounds had a lactam carbonyl peak at 1650 $cm.^{-1}$. Only chelidonine showed the presence of a hydroxyl group (3620 $cm.^{-1}$).

To show, chemically, that the two new alkaloids had the same substitution pattern, an attempt was made to convert methoxychelerythrine into sanguinaricine by demethylation using hydriodic acid followed by methylation of the polyphenol with diazomethane. Although this method failed, the conversion of oxymethoxychelerythrine to oxysanguinaricine was successful. The oxy-compound was prepared as previously described and refluxed in sodium-dried benzene with freshly sublimed aluminum chloride. This solution was then cooled, acidified and filtered. The filter-cake was dissolved in tetrahydrofuran (THF) and a solution of diazomethane in THF was added. After allowing time for methylation, the reaction product was purified by TLC and a few mg. of amorphous oxysanguinaricine were isolated. The identification of the product with the authentic compound was confirmed by lack of depression of a mixed melting point, TLC and by identical infrared spectra.

Now that the substitution patterns have been shown to be the same, it remained to show chemically that these alkaloids are substituted as proposed. This was to be done by first synthesizing the acids expected from a degradation of the alkaloids, then degrading the alkaloids and comparing the acids obtained.

This turned out to be somewhat more difficult than anticipated, but satisfactory results were finally obtained.

Degradation of methoxychelerythrine should yield, on the postulated structure, hemipinic and hydrastic acids, while sanguinaricine should yield

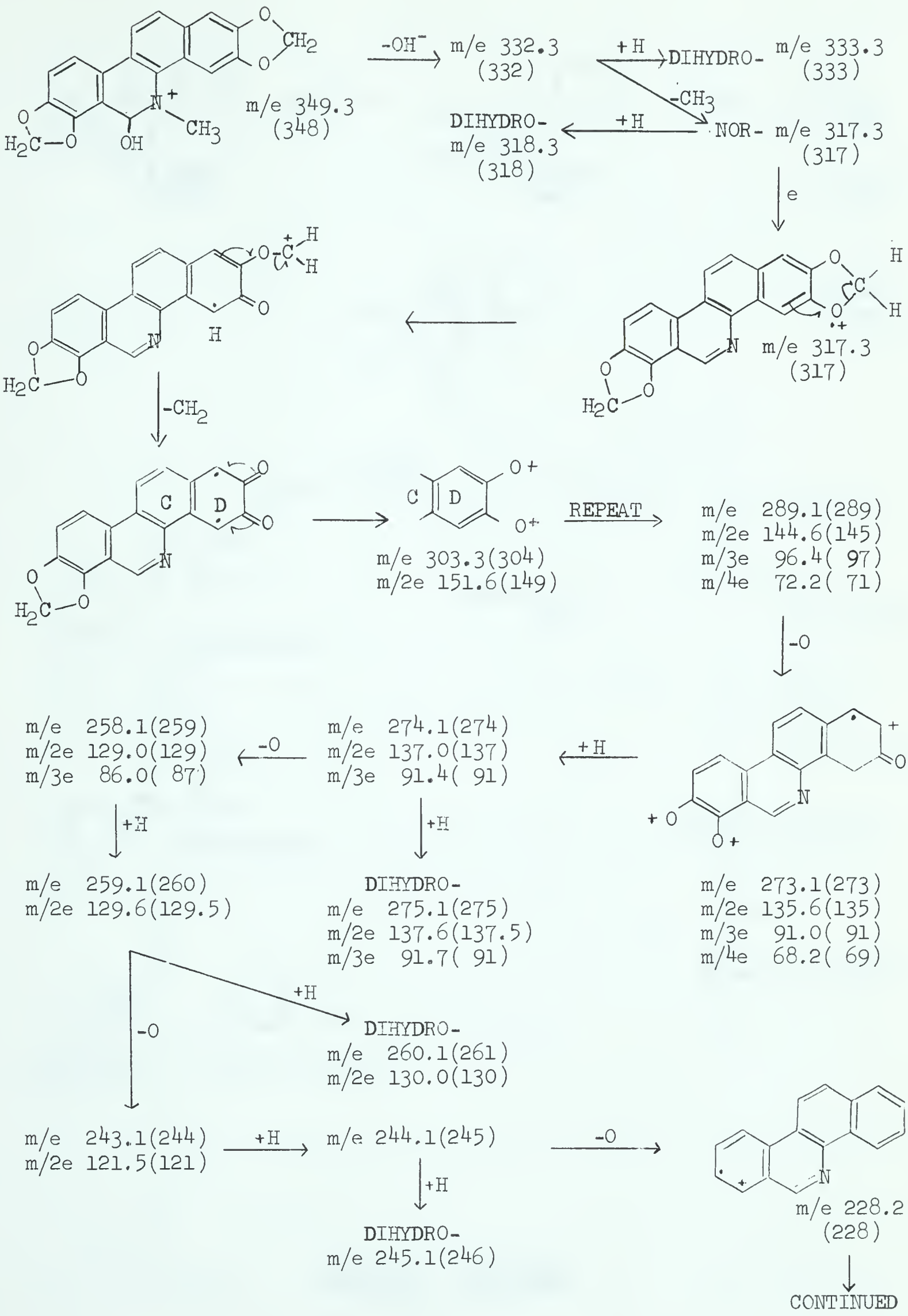
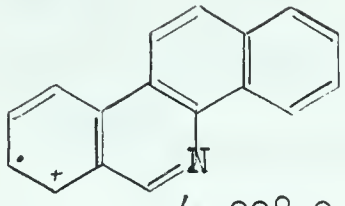


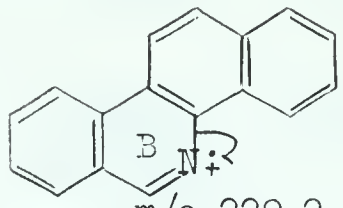
FIGURE IX. HYPOTHETICAL MASS SPECTRA FRAGMENTATION PATTERN FOR SANGUINARINE.

(EXPERIMENTAL VALUES ACCURATE TO ± 2 MASS UNITS)



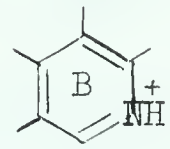
m/e 228.2
(228)

1) +H
2) e



m/e 229.2
(229)

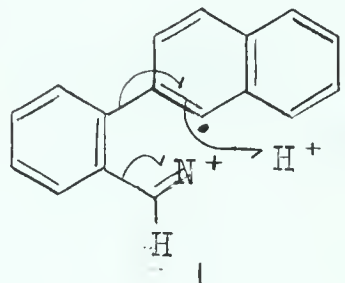
+H



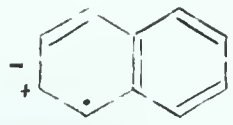
m/e 230.2
(230)

+H

DIHYDRO-
m/e 231.2
(231)



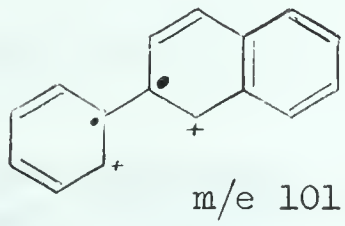
-CH=N



m/e 127.1(127)

+H

$CH_2=N^+$ m/e 28
(28)



m/e 101(101)

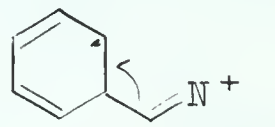
+H

m/e 202(202)

+H

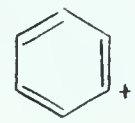
m/e 77(77)

m/e 127.1(127)



m/e 103(104)

-



m/e 77(77)

$HC\equiv N^+$ m/e 27(27)

+H

$CH_2=N^+$ m/e 28(28)

FIGURE IX. CONTINUED.

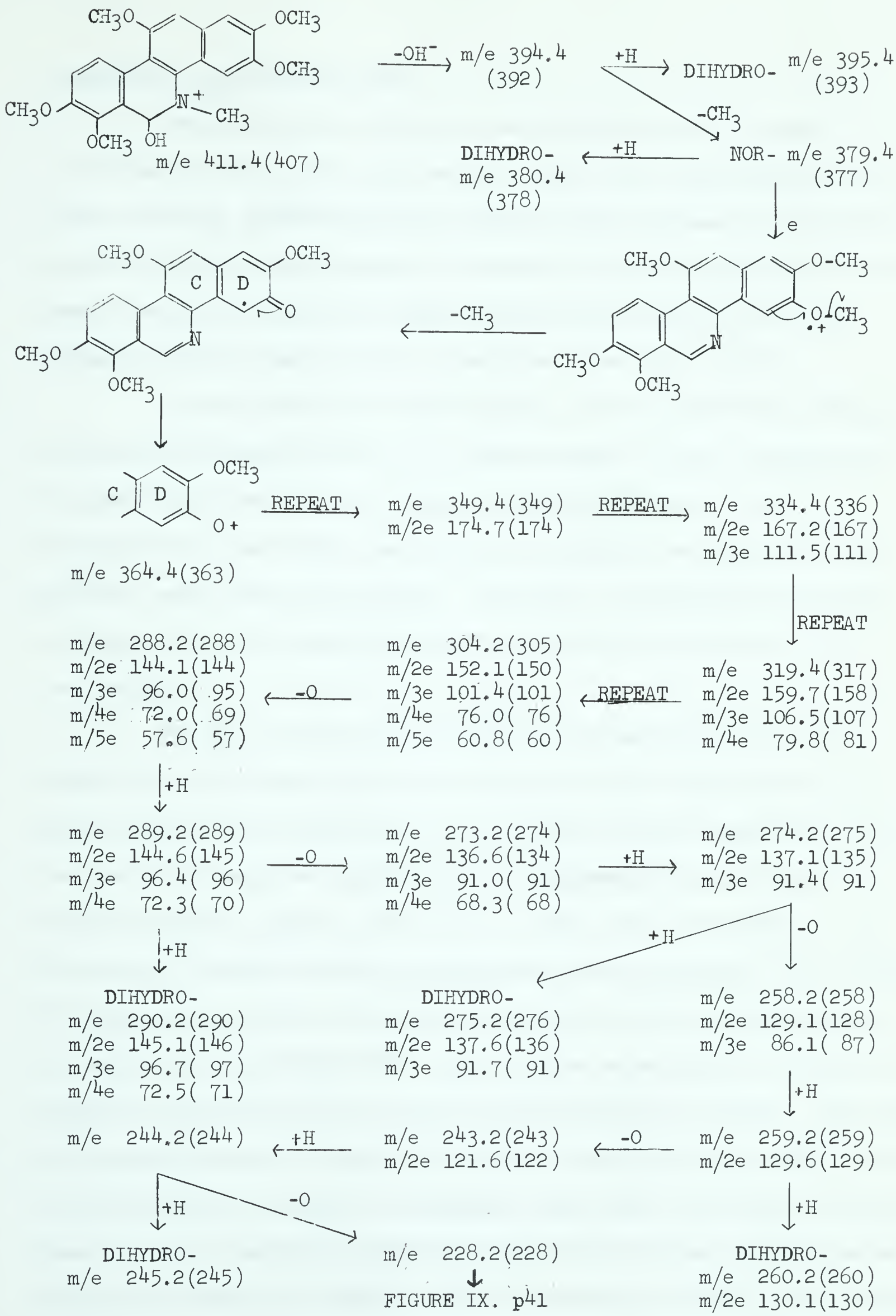


FIGURE X. HYPOTHETICAL MASS SPECTRA FRAGMENTATION PATTERN FOR SANGUINARICINE.

(EXPERIMENTAL VALUES ACCURATE TO ± 4 MASS UNITS)

m-hemipinic and hemipinic acids.

Hemipinic acid, isolated as the anhydride (shown by peaks in the infrared at 1850, 1770, 1250 and 1055 cm.$^{-1}$) was easily prepared from opianic acid (I, FIGURE XII) by the action of basic potassium permanganate. This acid, when chromatographed on a neutral silicagel G plate with an ethanol: ammonia:water elution mixture gave a yellow spot (Rf=0.20) on a blue background after spraying with a bromothymol blue spray reagent.

It was thought that once hydrastic acid was obtained, m-hemipinic acid could be prepared from it in a manner similar to the interconversion of oxymethoxychelerythrine to oxysanguinaricine. However, this is not essential as we have shown that methoxychelerythrine and sanguinaricine have the same substitution pattern. Therefore, if hydrastic acid can be obtained from methoxychelerythrine, the substitution pattern will have been proven.

For some obscure reason oxidation of piperonyl butoxide (II, FIGURE XII) which merely needs the conversion of two alkyl chains to acid groups, could not be achieved by any of several methods attempted. The only compound which could be isolated from these reactions was butyric acid. A lengthy synthesis, however, did lead to a small amount of hydrastic acid. This method involved a Doebner condensation between piperonal (III, FIGURE XII) and malonic acid to give piperonylacrylic acid (4,5-methylenedioxy-cinnamic acid) (IV, FIGURE XII). This acid was subsequently converted to the dihydro acid by the action of lithium aluminum hydride in ether. This method which was discovered by Brown (142) is rapid and simple and is quite valuable in the reduction of allylic or propargyl units. Up to this point the products were obtained in good yield. However, formylation of this compound was achieved only in low yields even though several methods were attempted, including one

with phosphorous oxychloride and N-methylformanilide. The best method found, was one based on the work of Stevens (143). Here the acid was dissolved in glacial acetic acid and refluxed with formalin and a trace of hydrochloric acid. The reaction mixture was then poured into water and extracted with chloroform. The lactone that was obtained was finally converted by the action of basic potassium permanganate, to hydrastic acid which shows carbonyl peaks at 1760 and 1710 cm. $^{-1}$ in the infrared and had an Rf=0.05.

Since the yield of hydrastic acid was very low, it was decided to degrade some chelidonine, by the method of Leete (80), and isolate the hydrastic and 3,4-methylenedioxyphthalic acids. Although the yield of acids here also was low, the two acids were detected by TLC, one of which appeared to be hydrastic acid (Rf=0.05) and the other was presumably 3,4-methylenedioxyphthalic acid (Rf=0.08). With this small amount of reference material at hand some methoxychelerythrine and sanguinaricine were similarly degraded and the acids isolated. Sanguinaricine gave hemipinic acid as well as another acid (Rf=0.52) presumably m-hemipinic whilst methoxychelerythrine gave hemipinic and hydrastic acids. Unfortunately not enough material was present to allow purification. However, TLC and infrared spectra of the somewhat impure samples showed that the desired acids had been obtained.

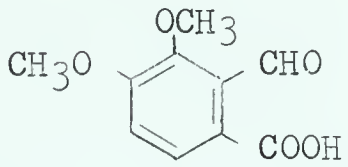
On completion of this primary phase of the programme the minor bases were then further investigated. A deep blue, almost non-fluorescent band near the base-line of the preparative TLC plates was removed, extraction from the silicagel and purified by crystallization from methanol. This colorless, crystalline material, m.p. 161-163°, was found to be identical with our fraction IIIa (Figure VII). The infrared spectrum showed that this compound contained a methylenedioxy group(s) (940 cm.$^{-1}$), a methoxyl group(s) (2850 cm.$^{-1}$) and carbonyl group (1650 cm.$^{-1}$). This alkaloid was shown to be identical

in all respects (TLC, infrared spectra and mixed melting point) to α-allocryptopine.

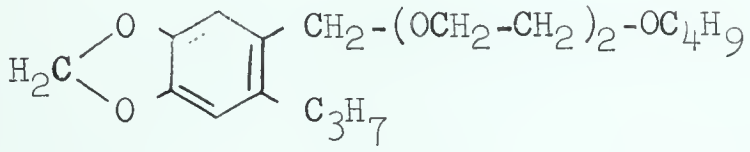
Extraction of the two blue fluorescent bands (Rf=0.55 and 0.48) showed these to be mixtures of oxysanguinarine and oxychelerythrine and of oxymethoxychelerythrine and oxysanguinaricine respectively. These were characterized by direct comparison with the authentic samples previously prepared.

Close to the top of the developed plate (Rf=0.88) there occured a band which did not initially show an ultraviolet fluorescence. This band was shown to be a mixture of the dihydro-derivatives of sanguinarine, chelerythrine, methoxychelerythrine and sanguinaricine. Dihydrosanguinarine and dihydrochelerythrine were isolated in small amounts while dihydromethoxychelerythrine and dihydrosanguinaricine were identified by TLC only. In conjunction with the discovery of these dihydro-alkaloids, work in this laboratory indicated that certain preliminary experiments with a limited amount of fresh plant material would indicate that the alkaloids are present in the plant as the dihydro-derivatives which are converted to the unsaturated bases during the normal collection and extraction procedures.

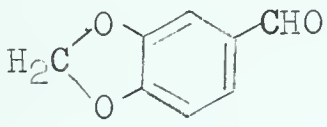
NO.		M.p.°C.	REFERENCE
I	OPIANIC ACID	150	60,79



II	PIPERONYL BUTOXIDE	B.p.°C. 180	60,79



III	PIPERONAL	37	60,79



IV	PIPERONYLACRYLIC ACID	240	79

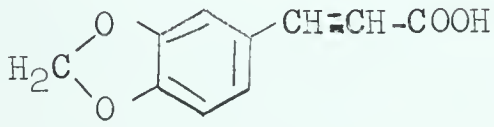
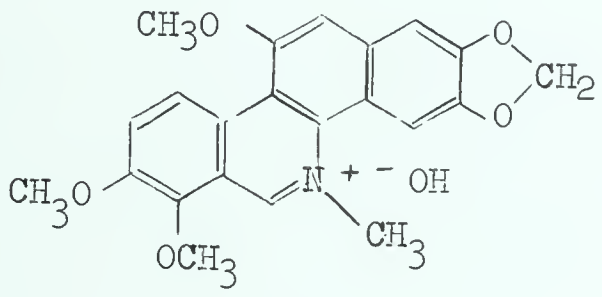


FIGURE XII. STRUCTURES OF MATERIALS USED IN THE SYNTHESES

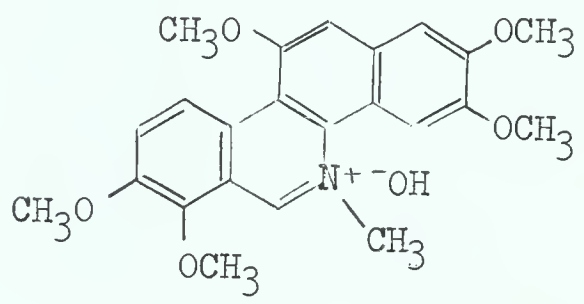
PART IV

CONCLUSIONS

During the course of this investigation eleven alkaloids have been isolated and at least six were detected by TLC. Of those isolated, four were 1,2-benzophenanthridine alkaloids (sanguinarine, chelerythrine, methoxychelerythrine, and sanguinaricine) the last two of which were previously unreported. The evidence available indicates that the structures of the new alkaloids are as shown in Figure XI.



METHOXYCHELERYTHRINE



SANGUINARICINE

FIGURE XI. STRUCTURES FOR METHOXYCHELERYTHRINE AND SANGUINARICINE.

This is the first time these two alkaloids have been reported and is also the first time benzophenanthridine alkaloids of the sanguinarine subgroup have been reported with five alkoxy substituents. Furthermore, the discovery of sanguinaricine is the first time a 1,2-benzophenanthridine alkaloid has been isolated that contains no methylenedioxy group.

In addition to these two new bases, dihydrosanguinarine and dihydrochelerythrine, have been isolated from this plant for the first time. Dihydromethoxychelerythrine and dihydrosanguinaricine were also detected. In addition, oxysanguinarine, oxychelerythrine, oxymethoxychelerythrine and oxysanguinaricine were also isolated which, with the exception of oxysanguinarine, is the first time these alkaloids have been isolated from

S. canadensis. These oxy-compounds resemble Slavik's (28) new sanguinaria alkaloids since they gave purplish-blue colors with acids. However, from the data available, it must be concluded that none of the newly isolated alkaloids correspond with those reported by Slavik.

Also isolated was α-allocryptopine and traces of the four nor-compounds were detected.

Berberine is not present and neither could the presence of coptisine and protopine be confirmed.

PART V

EXPERIMENTAL

A. GENERAL

1. THIN LAYER CHROMATOGRAPHY

Thin layer chromatography (TLC) was used extensively throughout the course of this investigation, both as a qualitative and as a preparative tool. The separating medium used was generally Silicagel G (G. Merck and Company, Darmstadt) although alumina (M. Woelm, via Alupharm Chemicals, New Orleans) was used occasionally The separating medium was applied to glass plates (20 cm. x 20 cm. for preparative work, 8 cm. x 10 cm. for qualitative work) by the method of Stahl (144) with a Desaga SII adjustable applicator (Brinkman Instruments Inc., Great Neck, N.Y.). Although the plates for qualitative work (0.25 mm. thick) could be prepared by making the slurry* with water, plates prepared in this manner for preparative work (1.0 mm. thick) cracked upon drying and became useless. However, by using 1,2-dimethoxyethane and water (2:8), plates were obtained which were satisfactory for preparative work (145). All preparative work was done using Silicagel G.

Occassionally it was found necessary to have non-acidic silicagel plates. These plates were prepared by a modified technique of Doepske (146), using a 0.1N sodium carbonate solution in place of the water in preparing the slurry. This allowed the detection of acidic materials by spraying with bromothymol blue indicator solution.

Development of the coated plate, after suitable activation and application of the test material, was by the "supersaturated chamber" technique (147). Various eluting solvent mixtures* were used, depending on the material and type of plate used. For preparative work only one solvent (chloroform: n-hexane: methanol:: 8:12:2) was used.

* See Appendix

Since the majority of the materials investigated were fluorescent under ultraviolet irradiation, this was the preferred means of detection. Following this, except in the case of preparative plates, the plates were sprayed* with the modified Dragendorff's reagent (148) and/or 5% ceric sulfate in 10% sulfuric acid to determine which fluorescent compounds were alkaloidal or organic matter respectively. The ceric sulfate spray has a universal application for all organic compounds and can give characteristic colorations at room temperature or a permanent brown-black color, on a white background when the plate has been heated for five minutes at 120° C. (145). As previously mentioned, when acidic compounds were suspected to be present, bromothymol blue was the spray used in conjunction with "non-acidic" plates.

The sample was applied to the preparative plates in a thin, continuous line 2 cm. from the bottom of the plate. The loading was 15-30 mg. per 20 cm. (1.0 mm.), corresponding to an approximate 1:1300 to 1:700 W:W relation between sample and medium respectively.

Following development of the plates, they were allowed to dry before the separated components were removed with a modification of the "microvacuum cleaner" method (149). The pure component was recovered from the silicagel by a micropercolation technique using chloroform:methanol: ammonia::10:10:1 as menstrum. The menstrum was then filtered, first through a sintered glass funnel of medium porosity and then through one of fine porosity, and the filtrate evaporated to dryness under reduced pressure.

Although the Rf values for the alkaloids will be reported they are of only relative importance because they were found to vary not only from day to day (150) (i.e. changing atmospheric conditions) but also

* See Appendix.

depended on whether the sample applied was pure or a mixture.

2. PAPER CHROMATOGRAPHY

This technique was used to help correlate some of the literature data with the experimental. The chromatogram was developed by the ascending solvent technique using butanol:water:acetic acid [10:3:1 (44) or 4:5:1 (41)] on Whatman number 1 chromatography paper (103). The position of the components on the chromatogram was detected, first by examination of their fluorescence under ultraviolet light, then by spraying with the modified Dragendorff's reagent (148) which gave a red-brown coloration with most alkaloids.

3. COLUMN CHROMATOGRAPHY

In the course of this investigation alumina (M. Woelm, via Alupharm Chemicals, New Orleans), silicagel BDH for adsorption chromatography (British Drug Houses, Poole, England) and silicagel for TLC (G. Merck and Company, Darmstadt) were used as the adsorbent. The alumina was used in a 100:1 ratio, the silicagel BDH in a 200:1 ratio and the TLC silicagel in a 300:1 ratio with the sample. While the first two adsorbents gave a fair separation of the components the third adsorbent (TLC silicagel) was found to be entirely unsatisfactory for adsorption chromatography due to its long development time (145).

In all cases the columns were packed by gravity in petroleum ether (b.p. 35-80°).

4. MELTING POINTS

All melting points were determined on a Leitz micro-Kofler block

(W.A. Carveth Ltd., Vancouver), using calibrated mercury thermometers.

5. SPECTRAL DATA

The infrared and ultraviolet spectra were recorded on Perkin Elmer D.B.21 and Unicam S.P.700 spectrophotometers respectively. Nuclear magnetic resonance spectra* were obtained from Varian Associates H.R.100 spectrophotometer using tetramethylsilane as the reference. The mass spectra** were obtained on a modified model of an M.S.2H spectrophotometer (Associated Electrical Industry Ltd.).

6. PLANT MATERIAL

The dried rhizomes and roots of Sanguinaria canadensis were obtained commercially from S.B. Penick and Company, New York.

B. EXTRACTION AND SEPARATION OF THE COMPONENTS OF S. CANADENSIS

1. EXTRACTION

One kilogram of the roots and rhizomes of Sanguinaria canadensis (Bloodroot) was ground to a fine powder and exhaustively extracted with hot methanol. The extract, which was first evaporated to a thick, syrupy mass, was then dissolved in 200 ml. of boiling glacial acetic acid. This solution was then poured into one liter of ice-cold water. After standing overnight in the refrigerator the mixture was filtered and the filtrate evaporated to dryness. This residue was dissolved in chloroform and shaken with water. Upon evaporation, the chloroform fraction yielded 55.6 g. of material (Fraction I) which gave a positive test for alkaloids. The aqueous

* Obtained through the courtesy of Mr. G. Bigam, Department of Chemistry, University of Alberta.

** Obtained through the courtesy of Mr. W. Duholke, Department of Chemistry,

phase was then extracted with butanol and upon evaporation of the butanol a few grams of non-alkaloidal material was obtained. This material was not further investigated. The alkaloidal fraction was subjected to a preliminary chemical work up but no significant separation was obtained. When this method of separation proved futile, a solvent that would give a good separation on the thick plates (1.0 mm.) was sought.

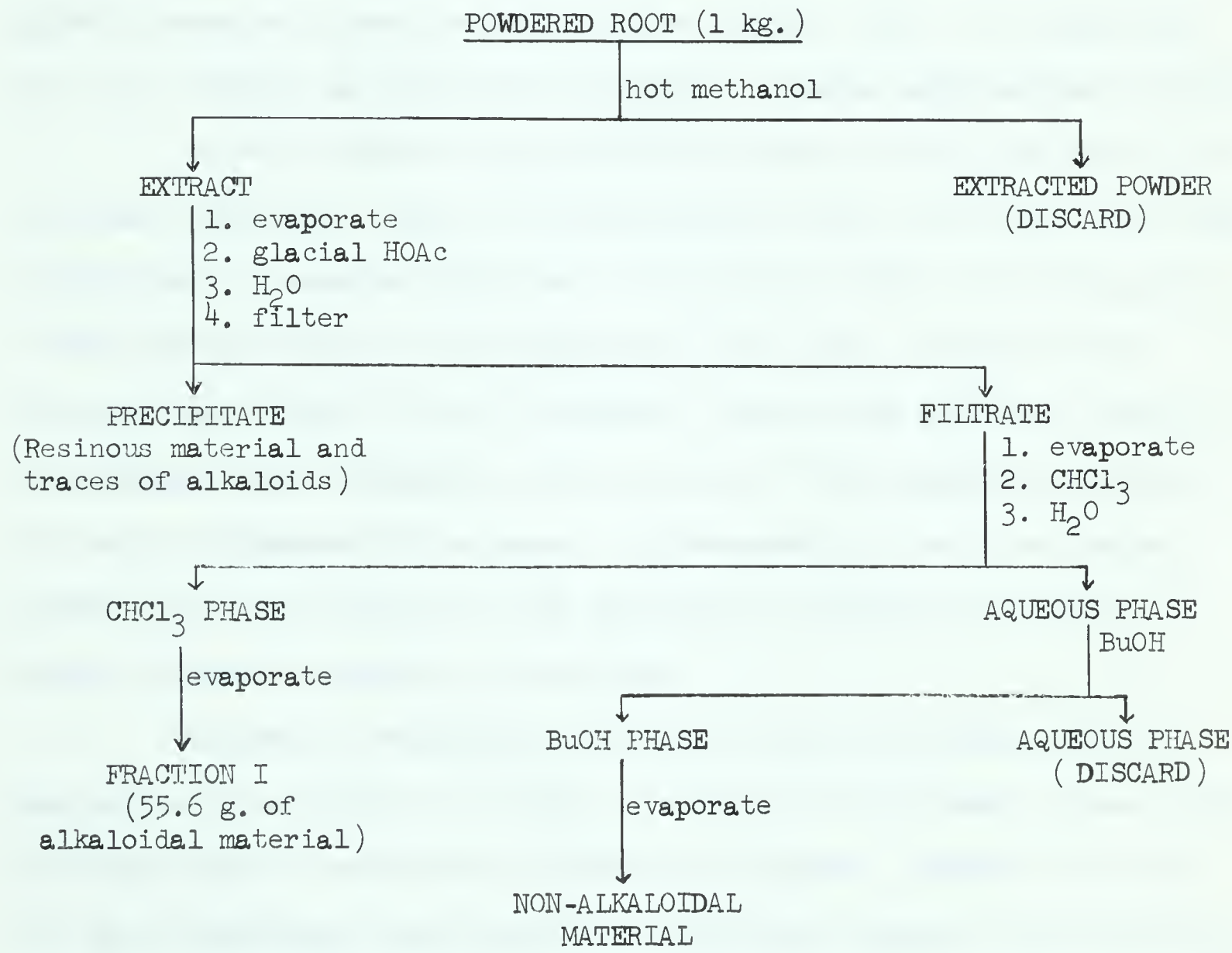


FIGURE VI. FLOW CHART OF CRUDE EXTRACTION

Various combinations of chloroform, n-hexane, methanol, diethylamine, ethylacetate, ammonia and glacial acetic acid were tried as solvent mixtures

for developing TLC plates to give a separation of the bases. After comparing over twenty solvents it was found that the solvent composed of chloroform: n-hexane:methanol::8:12:2 gave the best separation of the alkaloids.

Approximately 20 mg. of the alkaloidal sample was dissolved in a minimum volume of chloroform:methanol::1:1 and the sample was applied as described earlier. After the plates had been developed they were placed under an ultraviolet light and the six major zones were removed from the plate by means of a modified "microvacuum cleaner" (149). The alkaloids were then isolated as previously described. (General experimental techniques.)

By this method it was possible to obtain a few mg. of each of the four main alkaloids. (Two of the alkaloids were not investigated until later.) On the basis of reported color tests (26,39,58,151,152) and melting points it was evident that base 1 was sanguinarine and base 3 chelerythrine; however, bases 2 and4 did not correspond to any of the previously reported benzophenanthridine alkaloids, not even that of those alkaloids suspected of being benzophenanthridine bases. Unfortunately, in order to further examine these four alkaloids, more material was necessary and hence a faster method of obtaining it was sought.

Although it was known from earlier work that alumina (III-IV) was not sufficiently active to give a separation of the bases, it was hoped that this could be achieved by a more active alumina. Towards this end 547 mg. of fraction I was dissolved in chloroform (ethanol free) and placed on a column made from 50.5 g. of alumina (activity I). A fraction (260 mg.) which consisted of a mixture of bases 1-4 with traces of base 5 and 12 was eluted rapidly with chloroform. No other major fraction was obtained, as the remaining components tended to "trail" together. Although this column did greatly purify the benzophenanthridine fraction, it held no advantage over the alumina (activity III-IV). The material obtained from

the alumina column was then subjected to a silicagel (BDH) column with an adsorbent to sample ratio of 200:1. This column also proved ineffective in separating the bases 1-5. Since separation on a silicagel TLC plate was quite good it was decided to try a silica column using TLC silica. 120 mg. of sample were placed on 35.5 g. of TLC silicagel in a glass column. Here again the desired separation was not obtained and the column chromatography technique was abandoned since it had consistently failed to separate the alkaloids.

The next method used was designed to exploit the properties of the two known alkaloids in the mixture (FIGURE VII). 15.79 g. of the alkaloidal mixture (I) was dissolved in 70 ml. of glacial acetic acid and then diluted to 5% with water. This solution was cooled and filtered and a brown, non-alkaloidal material (II) was filtered out. The mother liquors were basified with ammonia to pH9, cooled and filtered. An alkaloid (IIIa), which later was shown to be a α-allocryptopine, was then extracted with chloroform from the aqueous mother liquors. The aqueous mother liquors contained only traces of alkaloids and hence were discarded. The precipitate (IV) was suspended in ether and then evaporated to dryness to remove any traces of water. It was again suspended in ether and filtered. The residue (V) was mainly non-alkaloidal in nature, but did contain traces of bases 1-5 and 12. However, fraction VI, the ether extract, now consisted chiefly of bases 1-4, although base 5 and 12 and other non-alkaloidal materials were present in small amounts. Fraction VI was finally separated by the use of preparative TLC. Initially, 410 mg. of VI was applied (18 plates) and bases 1-5 and 12 were recovered in a relatively pure state. The recovered alkaloids represented about 70% of the applied material, and as such represented a much higher yield than when the crude alkaloidal extract

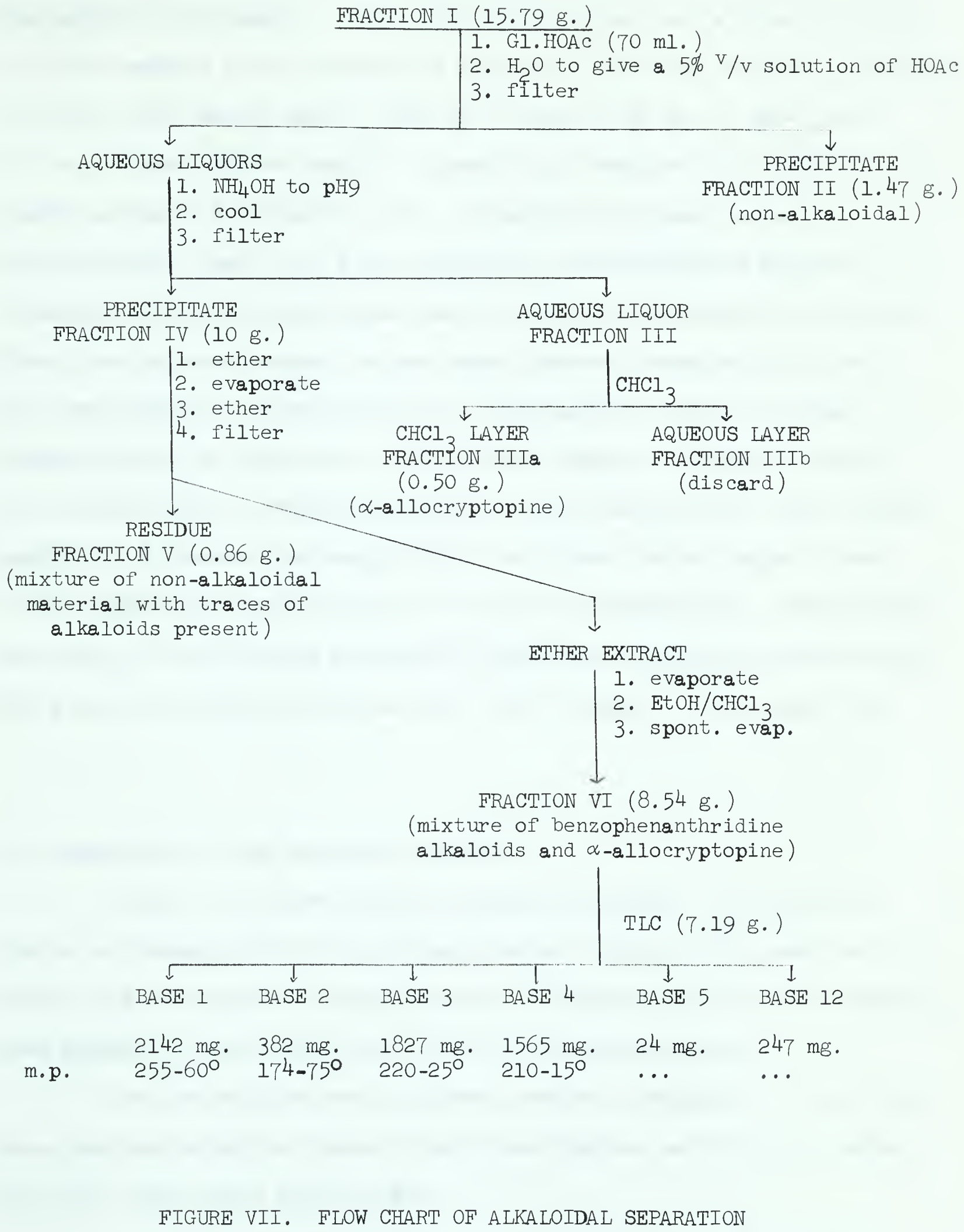


FIGURE VII. FLOW CHART OF ALKALOIDAL SEPARATION

was applied to the plates. Since this method worked well a total of 7.19 g. of VI was applied to 240 preparative TLC plates. 2142 mg. of base 1, 382 mg. of base 2, 1827 mg. of base 3, 1565 mg. of base 4, 24 mg. of base 5 and 247 mg. of base 12 were obtained. Bases 1 to 4 were the ones first investigated and bases 5 and 12 were not scrutinized until later in the investigation. Bases 1 to 4 were purified by crystallization and their infrared, ultraviolet and nuclear magnetic resonance spectra were obtained. These four bases all showed the same major spectral characteristics and all were similar to xanthofagarine (5), although none had the spectral characteristics of chelidonine (L. Light and Company). Elemental analysis were obtained (Dr. F. Pascher, Mikroanalytisches Laboratorium, Bonn, Germany and/or Dr. C. Daessle, Montreal, Canada) and these further supported our belief that these alkaloids were not related to chelerythrine. Sanguinarine was readily identified when a commercial sample of sanguinarine was obtained . The presence of chelerythrine was also shown by paper chromatography (41).

C. EXAMINATION OF THE ISOLATED COMPONENTS

Bases 1 to 4 were treated similarly throughout. The method of choice for preparation of the oxy-compounds was found, in all cases, to be method iv as discussed for oxysanguinarine. Similarly, the dihydro-compounds were prepared by the method described for dihydrosanguinarine.

For the sake of brevity, clarity and easy comparison, the physical data compiled during the course of this investigation are listed in tables IV to VII and figures XIII and XIV.

All Rf values recorded are for silicagel TLC using chloroform:

n-hexane:methanol (8:12:2) unless otherwise stated.

1. (a) SANGUINARINE

By means of comparative paper chromatography (94) it was suspected that base 1 was sanguinarine. Base 1 (Rf=0.79, orange fluorescence) melts at 265-67° [239-42 (26,20), 266 (28,94)] when crystallized from 95% ethanol. Labat's test for methylenedioxy groups, carried out by dissolving a few crystals of the material in a freshly prepared solution of gallic acid in concentrated sulfuric acid, was positive (appearance of a blue color). This was substantiated by a peak at 945 cm.$^{-1}$ in the infrared spectrum.

The nuclear magnetic resonance (NMR) spectrum showed the following peaks: 7.25τ (3H,N-methyl), 6.31-5.98τ (2H, CH_2 from ethanol), 8.97-8.83τ (3H, CH_3 from ethanol), 3.95τ (2H, methylenedioxy-ring D), 3.89τ (2H, methylenedioxy-ring A), and 6 aromatic protons between 3.13τ and 2.19τ. There was also a single proton peak at 4.52τ, which was assigned to the non-aromatic proton at C_9—analogous to the carbinolamine structure for berberine—since it is at too high a field to be an aromatic proton. Sanguinarine, when vacuum sublimed at 220°, loses the O-ethyl group as shown by the loss of the NMR peaks at 6.31-5.98τ and 8.97-8.83τ.

The ultraviolet absorption maxima, taken in purified ethanol (95%), were obtained and, along with the calculated log.ϵ,are recorded in table V. Although no shift occurred in alkaline solution, the addition of acid caused a substantial bathochromic shift.

Base 1 readily formed a red-orange chloride, m.p. 269-71° [272-73° (28)], either by addition of ether/hydrochloric acid to a chloroform solution or by dissolving the material in concentrated hydrochloric acid and then diluting with water.

The pseudocyanide was prepared by dissolving 25 mg. of base 1 in a warm chloroform/methanol (1:1) solution. To this warm solution was added warm, aqueous potassium cyanide (15 mg. in 1 ml.), and the mixture was boiled for fifteen minutes. The methanol and chloroform were removed under vacuum, and the aqueous solution was filtered. The collected solid was recrystallized from acidified methanol and dried. The buff-colored pseudocyanide melted at 239-42° [237-38° (101) 242-43° (28)].

This data provided strong proof that base 1 was sanguinarine. This was subsequently proven beyond a doubt by an infrared spectrum of, and a mixed melting point with an authentic sample of sanguinarine.

(b) NORSANGUINARINE

This compound melts at 261-64° [279-80° (38)] and is easily obtained by vacuum sublimation of sanguinarine chloride at 240°. It is also obtained during the preparation of oxysanguinarine if the reaction was allowed to continue for longer than one hour.

The infrared and ultraviolet spectra showed very little change from that of sanguinarine, however the NMR spectrum is void of peaks at 7.25 τ (3H, N-methyl), 6.31-5.98 τ (2H, CH_2 from ethanol) and at 8.97-8.83 τ (3H, CH_3 from ethanol).

Elemental analysis of resublimed norsanguinarine gave:
Found: C, 71.86%; H, 3.55%; N, 4.43%; O, 20.16% (by difference); molecular weight, 347.
Calculated for $C_{19}H_{11}O_4N$: C, 71.92%; H, 3.49%; N, 4.41%; O, 20.18%; molecular weight, 317.3.

In order to determine the molecular weight with more precision, a mass spectrum of sublimed sanguinarine was obtained. In this instance

the molecular weight was found to be 348 (calculated 349.3).

(c) OXYSANGUINARINE

(i) 47 mg. of sanguinarine were dissolved in 10 ml. of water and a hot solution consisting of 200 mg. of potassium ferricyanide and 90 mg. of potassium hydroxide in 5 ml. of water was added. A white precipitate formed immediately, however the mixture was warmed for a further fifteen minutes on a steam bath. The mixture was cooled and extracted with chloroform. Upon evaporation of the chloroform and crystallization of the residue, 22.5 mg. of a compound, m.p. 355-56° [356-58° (28), 360-61° (26,42)], were obtained. The infrared spectrum showed the characteristic peak at 1650 $cm.^{-1}$ (lactam carbonyl).

(ii) 25 mg. of sanguinarine was refluxed for 24 hours in a strong solution of potassium hydroxide. Although some oxysanguinarine (35,37) was obtained this method was discarded as unsuitable due to the low yield.

(iii) 50 mg. of sanguinarine were dissolved in 1 ml. of hot glacial acetic acid and diluted with 8 ml. of hot water. Potassium hydroxide (900 mg.) was added to bring the pH to near neutrality and a hot solution containing 200 mg. of potassium ferricyanide and 400 mg. of potassium hydroxide in 5 ml. of water was added. The whole was then heated on a steam bath for one hour, cooled and extracted with chloroform. The chloroform was washed several times with 5% acetic acid followed by saturated sodium bicarbonate

and finally with water. The chloroform was then evaporated and 12 mg. of oxysanguinarine (contaminated with some norsanguinarine) was obtained.

(iv) 22 mg. of sanguinarine were dissolved in 2 ml. of dimethylsulfoxide (DMSO), 2 ml. of water were added and the solution was heated on a water bath. A warm solution containing 200 mg. of potassium hydroxide and 200 mg. of potassium ferricyanide in 3 ml. H_2O and 3 ml. DMSO was added and the resulting solution was refluxed for one hour. After cooling, the solution was extracted with chloroform which was in turn washed with 5% acetic acid. After this removal of the remaining starting material (lack of color in the aqueous phase) the chloroform was washed twice with a saturated solution of sodium bicarbonate and once with water. The chloroform was evaporated and the residue crystallized from ethanol and 19.5 mg. of oxysanguinarine was obtained. Although there was still a small amount of unreacted starting material present, any increase in reflux time tended to lower the yield of oxysanguinarine by formation of norsanguinarine. This was shown to occur when under identical reaction conditions 50 mg. of sanguinarine were refluxed for three hours. 26 mg. of oxysanguinarine and 22 mg. of norsanguinarine were isolated. This type of behavior was also noted with all the other benzophenanthridine alkaloids studied, one hour being the optimal maximum time.

(d) DIHYDROSANGUINARINE

41.5 mg. of sanguinarine were dissolved in 5 ml. of methanol and 40 mg. of sodium borohydride were added. The reaction, marked by strong effervescence and change to a colorless solution, is extremely rapid, being complete in a matter of seconds. The colorless solution was evaporated "in vacuo" to dryness and the residue was extracted with chloroform containing ammonia and 40 mg. of dihydrosanguinarine, m.p. 187-90° [188-90 (42), 192 (38)], was crystallized from basic methanol (i.e. containing traces of ammonia).

An infrared spectrum showed only slight differences from the spectrum of the base. However, the ultraviolet spectrum, which showed no shift when compared to the spectrum of the base, generally showed a higher log.ε value (cf. TABLE V).

Although there was no shift in alkaline solution, the addition of acid again caused a bathochromic shift.

2. (a) CHELERYTHRINE

Base 3 (Rf=0.68, yellow fluorescence), m.p. 216-18° [207-10° (28,84,140), 239-40° (45), 257-59° (84), 282-83° (28)], gave a positive Labat's test for a methylenedioxy group. From its physical data (IR, UV and NMR spectra and m.p.) and by chromatographic comparison (94) this alkaloid was shown to be chelerythrine.

(b) NORCHELERYTHRINE

Norchelerythrine, m.p. 207-09° [221-22° (45), 212-14 (28)], is obtained by vacuum sublimation of the yellow chloride at 200°.

Analysis of the sublimed sample gave:

Found: C, 71.34%; H, 4.65%; N, 4.16%; O, 19.85% (by difference); OCH_3,

16.36%; molecular weight, 362.

Calculated for $C_{20}H_{15}O_4N$: C, 72.06%; H, 4.54%; N, 4.20%; O, 19.20%; $(OCH_3)_2$, 18.62%; molecular weight, 333.3.

3. (a) METHOXYCHELERYTHRINE

When crystallized from 95% ethanol, base 2 (Rf=0.72, deep red fluorescence) melts at 176-77° and gives a positive Labat's test. Its salts are a rust color.

(b) NORMETHOXYCHELERYTHRINE

Sublimation of the chloride salt, "in vacuo", at 190° gave nor-methoxychelerythrine, m.p. 200-02°: Analysis of the sublimed material gave: Found: C, 69.36%; H, 4.89%; N, 3.89%; O, 21.86% (by difference); OCH_3, 22.64%; molecular weight, 405.

Calculated for $C_{21}H_{17}O_5N$: C, 69.41%; H, 4.72%; N, 3.85%; O, 22.02%; $(OCH_3)_3$, 25.62%; molecular wieght, 363.4.

4. (a) SANGUINARICINE

Base 4 (Rf=0.62, salmon fluorescence) melts at 211-12° when crystallized from 95% ethanol and gives a negative Labat's test. Its salts are bright yellow.

(b) NORSANGUINARICINE

Nor-base 4, m.p. 169-71°, was obtained by subliming the chloride "in vacuo" at 200°. Analysis of the sublimed sample gave:

Found: C, 69.59%; H, 5.66%; N, 3.62%; O, 21.13% (by difference); OCH_3, 39.88%; molecular weight, 401.

Calculated for $C_{22}H_{21}O_5N$: C, 69.64%; H, 5.58%: N, 3.69%; O, 21.09%;

$(OCH_3)_5$, 40.98%; molecular weight 379.4.

As was the case for sanguinarine, a mass spectrum was obtained, of the sublimed base, to see if a more accurate determination of the molecular weight was possible. Although a poorly resolved spectra was obtained, the parent peak was found at 407, while the calculated molecular weight is 411.4.

5. ATTEMPTED CONVERSION OF METHOXYCHELERYTHRINE TO SANGUINARICINE

After 41 mg. of methoxychelerythrine had been refluxed for two hours in 5 ml. of hydriodic acid, 20 ml. of sodium-dried toluene were added and refluxing was continued for a further four hours. The solution was cooled, basified with ammonia and extracted with chloroform. The chloroform was evaporated and the residue (8.6 mg.) was dissolved in tetrahydrofuran (THF). A solution of diazomethane in the THF was added to the previous solution and allowed to react for twelve hours.

The THF was evaporated under vacuum and the residue was extracted with chloroform. When the chloroform was evaporated, 2.2 mg. of an unidentified, amorphous mass was obtained. No sanguinaricine was isolated, nor could any be detected by TLC.

6. CONVERSION OF OXYMETHOXYCHELERYTHRINE TO OXYSANGUINARICINE

A mixture of 25 mg. of oxymethoxychelerythrine dissolved in 10 ml. of sodium-dried benzene and 200 mg. of freshly sublimed aluminum chloride was refluxed for one hour. The solution was cooled and acidified with 10 ml. of dilute hydrochloric acid. The resultant precipitate (7.8 mg.) was collected by microfiltration and then redissolved in THF. To this a solution of diazomethane in THF was added and allowed to stand for six hours.

TABLE IV

MELTING POINTS OF THE 1,2-BENZOPHENANTHRIDINE ALKALOIDS AND SOME DERIVATIVES

COMPOUND	EXPERIMENTAL m.p. °C.	LITERATURE m.p. °C.	REFERENCE
CHELIDONINE	134-35	135-36	26
NOR-	---	146	55,91
OXY-	---	>285	20,26
HYDROCHLORIDE	202-04	204-05	26,57
O-ACETYL-	---	161-63,184-86	20,26
HOMOCHELIDONINE	---	182	26,58
METHOXYCHELIDONINE	---	221	20,26
HYDROCHLORIDE	---	237-38	20
O-ACETYL-	---	147	20
AVICINE	---	---	---
OXY-	---	275-77	37
DIHYDRO-	---	211-13	37
PSEUDOCYANIDE	---	>340	37
ACETATE	---	160	37
CHELERYTHRINE	216-18	207-10	28,84,140
		239-42	45
		257-59	84
		282-83	28
NOR-	207-08	221-23,212-14	28,45
OXY-	189-92	199-201	45
DIHYDRO-	166-67	160-62;166-67	42,45
HYDROCHLORIDE	203-05	202-03,213-14	28,84
PSEUDOCYANIDE	258-60	229-33	45
		258	44
		260-61	28,41,84
NITRATE	---	238	45
METHOXYCHELERYTHRINE	176-79	---	---
NOR-	200-02	---	---
OXY-	190-93	---	---
DIHYDRO-	88-91	---	---
HYDROCHLORIDE-	200-03	---	---
SANGUINARINE	265-67	239-42	20
		265-67	28,94
NOR-	261-64	279-80	38
OXY-	255-56	356-58	28
		360-61	42
DIHYDRO-	187-90	188-89	42
		192	38
HYDROCHLORIDE	269-71	272-73	28
PSEUDOCYANIDE	239-42	237-38	101
		242-43	28

TABLE IV CONTINUED

COMPOUND	EXPERIMENTAL m.p. °C.	LITERATURE m.p. °C.	REFERENCE
ACETATE	---	225	38
SANGUINARICINE	211-12	---	---
NOR-	169-71	---	---
OXY-	245-47	---	---
DIHYDRO-	149-52	---	---
HYDROCHLORIDE	122-24	---	---
PSEUDOCYANIDE	217-20	---	---
XANTHOFAGARINE	---	277-78	36
OXY-	---	284-85	35
DIHYDRO-	---	221-23	35
HYDROCHLORIDE	---	285-86	35
PSEUDOCYANIDE	---	234	35
ACETATE	---	255-60	35

TABLE V

ULTRAVIOLET SPECTRAL DATA FOR THE 1,2-BENZOPHENANTHRIDINE ALKALOIDS

	CHELIDONINE		AVICINE		CHELERYTHRINE					
	BASE		DIHYDRO- (37)		BASE		DIHYDRO-		NOR-	
	λ max. mμ	log. ε	λ max. mμ	log. ε	λ max. mμ	log. ε	λ max. mμ	log. ε	λ max. mμ	log. ε
NEUTRAL	211.0	4.50	232	4.33	210.1	4.33	211.0	4.40	241.0	4.58
	238.1	3.96	278	4.50	227.3	4.45	227.8	4.52	256.4	4.54*
	289.1	3.78	322	4.60	282.5	4.54	281.7	4.61	266.4	4.53
					316.5	4.09	318.6	4.17	278.0	4.52*
									316.5	4.10
									384.2	3.89
									400.0	3.90
									441.0	4.04
ACIDIC	211.9	4.52			214.6	4.30	214.6	4.35*	222.0	4.54
	242.7	3.92			225.3	4.26*	232.6	4.49	241.0	4.56*
	290.7	3.86			248.2	4.34*	256.4	4.42*	278.0	4.52*
					270.3	4.48	263.1	4.52	305.2	4.08
					279.5	4.46*	273.3	4.57	339.5	4.06
					326.9	4.38	277.0	4.49*	362.3	3.88*
					340.2	4.22	290.7	4.18*	407.5	3.93
					395.4	3.65	303.0	4.28	444.5	4.03
					429.4	3.48*	316.5	4.36		
							333.3	4.13		
							350.9	4.20		

* Inflection

NOTE: Ultraviolet spectra for norchelerythrine, normethoxychelerythrine and norsanguinaricine were obtained from a Cary Model 14M spectrophotometer.

TABLE V CONTINUED

METHOXYCHELERYTHRINE

	BASE		DIHYDRO-		NOR-	
	λ max. mμ	log. ε	λ max. mμ	log. ε	λ max. mμ	log. ε
NEUTRAL	212.8	4.37*	206.3	4.32*	244.0	4.66*
	229.4	4.46	228.3	4.39	272.2	4.56
	279.5	4.50	277.8	4.38	278.0	4.53*
	326.9	4.15	325.7	4.04	314.2	4.00
					327.0	4.37
					343.0	3.96
					407.0	3.36
					463.0	4.05
ACIDIC	213.7	4.30	207.0	4.24*	215.0	4.57*
	233.7	4.37	237.0	4.35	235.5	4.60
	243.7	4.41	242.7	4.30*	269.0	4.54
	273.3	4.50*	264.6	4.28*	278.0	4.49*
	279.5	4.53	271.8	4.32	333.5	4.15
	342.5	4.39	284.1	4.11	365.0	2.60*
	410.0	3.53	294.1	3.93*	400.0	3.69
	467.5	3.66	314.5	3.98*	455.0	3.93
			325.7	4.09		
			337.9	4.08		
			353.4	4.07		

SANGUINARINE

	BASE		DIHYDRO-		NOR-	
	λ max. mμ	log. ε	λ max. mμ	log. ε	λ max. mμ	log. ε
NEUTRAL	211.4	4.31*	212.8	4.40*	212.8	4.31
	235.9	4.41	235.9	4.51	243.9	4.56
	284.9	4.44	282.5	4.51	282.5	4.51
	322.6	4.10	322.6	4.14	330.1	4.18*
					384.6	3.49
					400.0	3.48*
ACIDIC	215.5	4.25	214.6	4.33*	215.5	4.33
	235.9	4.27*	238.1	4.50	243.9	4.45
	248.8	4.32*	250.0	4.40*	273.3	4.47
	257.7	4.35	264.5	4.51	324.7	4.39
	274.8	4.41	274.8	4.38	390.8	3.57
	329.0	4.33	284.1	4.23*	471.9	3.48
	393.8	3.52	294.1	4.12*		
	471.9	3.59	306.8	4.13		
			320.6	4.28		
			337.9	4.13		
			353.4	4.16		

* Inflection

TABLE V CONTINUED

SANGUINARICINE

	BASE		DIHYDRO-		NOR-	
	λ max. mμ	log. ε	λ max. mμ	log. ε	λ max. mμ	log. ε
NEUTRAL	208.3	4.32*	205.8	4.39*	247.0	4.65
	228.3	4.45	227.3	4.53	272.0	4.64*
	277.8	4.52	274.8	4.60	298.0	4.28*
	329.0	4.18	326.8	4.26	316.5	4.17
					328.2	4.16*
					347.5	4.16
					392.5	4.06*
					406.5	4.08
					468.0	4.17
ACIDIC	215.5	4.31	208.3	4.31*	206.5	4.66*
	229.4	4.33*	229.4	4.51	244.0	4.65*
	245.1	4.40	241.6	4.46*	284.0	4.31*
	271.8	4.49*	264.6	4.49*	327.0	4.23
	279.5	4.53	271.8	4.56	338.0	4.21*
	342.5	4.39	284.1	4.15*	368.5	4.05
	413.3	3.43*	297.7	3.96	400.0	4.12
	469.7	3.59	312.5	4.11	455.5	4.13
			326.8	4.30		
			337.9	4.31		
			353.4	4.38		

XANTHOFAGARINE

	BASE (36)		DIHYDRO- (35)	
	λ max. mμ	log. ε	λ max. mμ	log. ε
NEUTRAL	229.9	4.55	228	4.29
	280.1	4.54	278	4.54
	310.6	4.27	311	4.61
ACIDIC	220.8	4.29		
	237.0	4.31		
	271.8	4.61		
	291.5	4.61		
	300.3	4.58		
	328.9	4.59		
	387.6	4.01		

* Inflection

TABLE VI

NMR SPECTRAL DATA FOR THE 1,2-BENZOPHENANTHRIDINE ALKALOIDS

FUNCTION	CHELERYTHRINE	METHOXY-CHELERYTHRINE	SANGUINARINE	SANGUINARICINE	XANTHOFAGARINE (36)
$N\text{-}CH_3$	7.250	7.312	7.247	7.233	8.13
$\text{-}O\text{-}CH_2\text{-}$	6.305-5.964	6.346-6.051	6.310-5.983	6.413-5.813	---
$\text{-}CH_3$	8.979-8.826	9.026-8.881	8.967-8.832	9.028-8.883	5.42($\text{-}OCH_3$ non ar.)
$A\text{-}O_2CH_2\text{-}$	---	---	3.887	---	---
$D\text{-}O_2CH_2\text{-}$	3.972	3.970	3.950	---	4.18
$A\text{-}OCH_3$	C_7 6.035	C_7 6.050	---	C_7 6.060	C_7 6.06
	C_8 6.102	C_8 6.080	---	C_8 6.080	C_6 6.18
$D\text{-}OCH_3$	---	---	---	C_2, or 3, 6.00	---
	---	---	---	C_2, or 3, 5.94	---
$C_4\text{-}OCH_3$	---	6.050	---	6.060	---
					7H, 3.0-1.7
C_9 H	4.316	4.353	4.515	4.338	---
C_3 H	3.035&2.962	3.367	3.132&2.947	3.356	---
C_4, H	2.900	2.894	2.892	2.878	---
C_1, H	2.600&2.520	2.609&2.514	2.643,2.581 &2.560	2.573&2.486	---
C_6 H	2.444	2.303	2.497	2.322	---
C_4 H	2.358,2.336 &2.272	---	2.341&2.289	---	---
C_5 H	2.196	1.604&1.419	2.195	1.589&1.501	---

ALL VALUES RECORDED IN TAU (τ) UNITS.

(FOR NUMBERING SEE I, TABLE III)

FIGURE XIV

NUCLEAR MAGNETIC RESONANCE SPECTRA OF THE 1,2-BENZOPHENANTHRIDINE ALKALOIDS

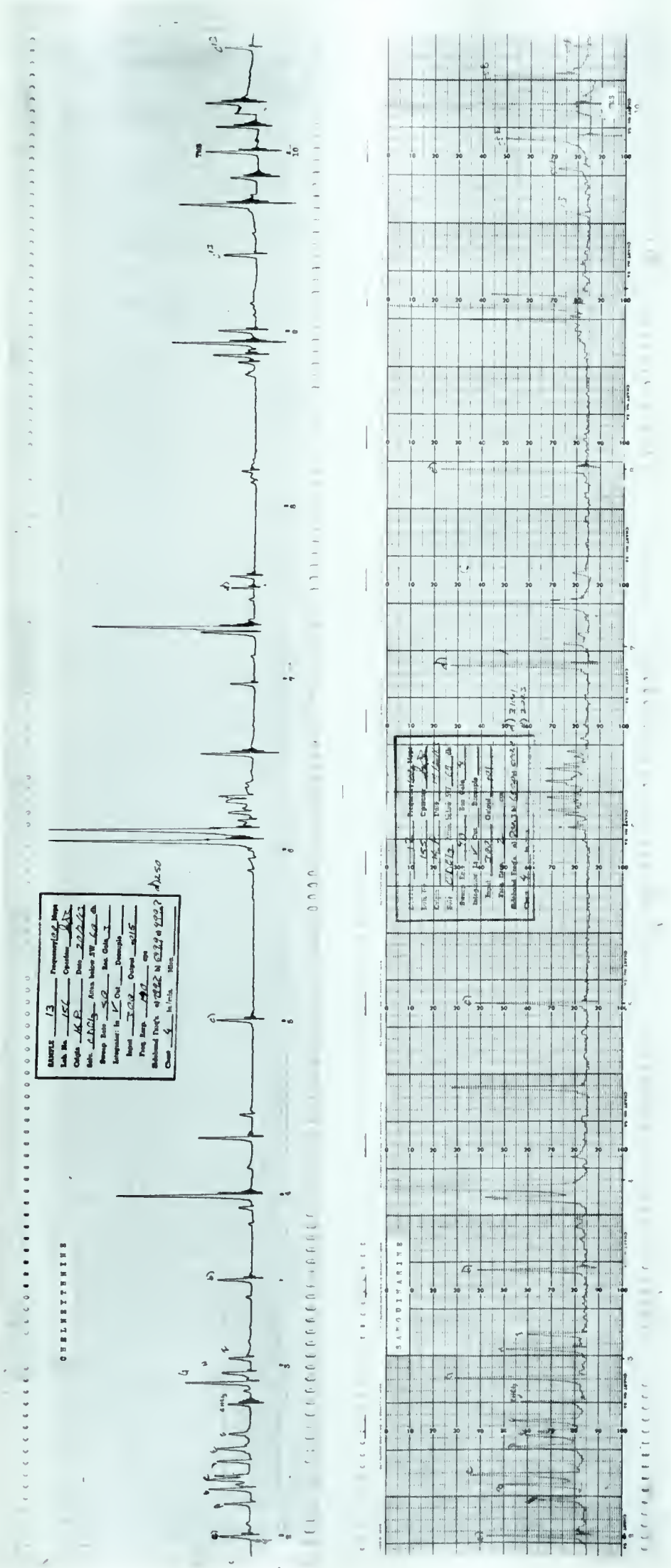


FIGURE XIV CONTINUED

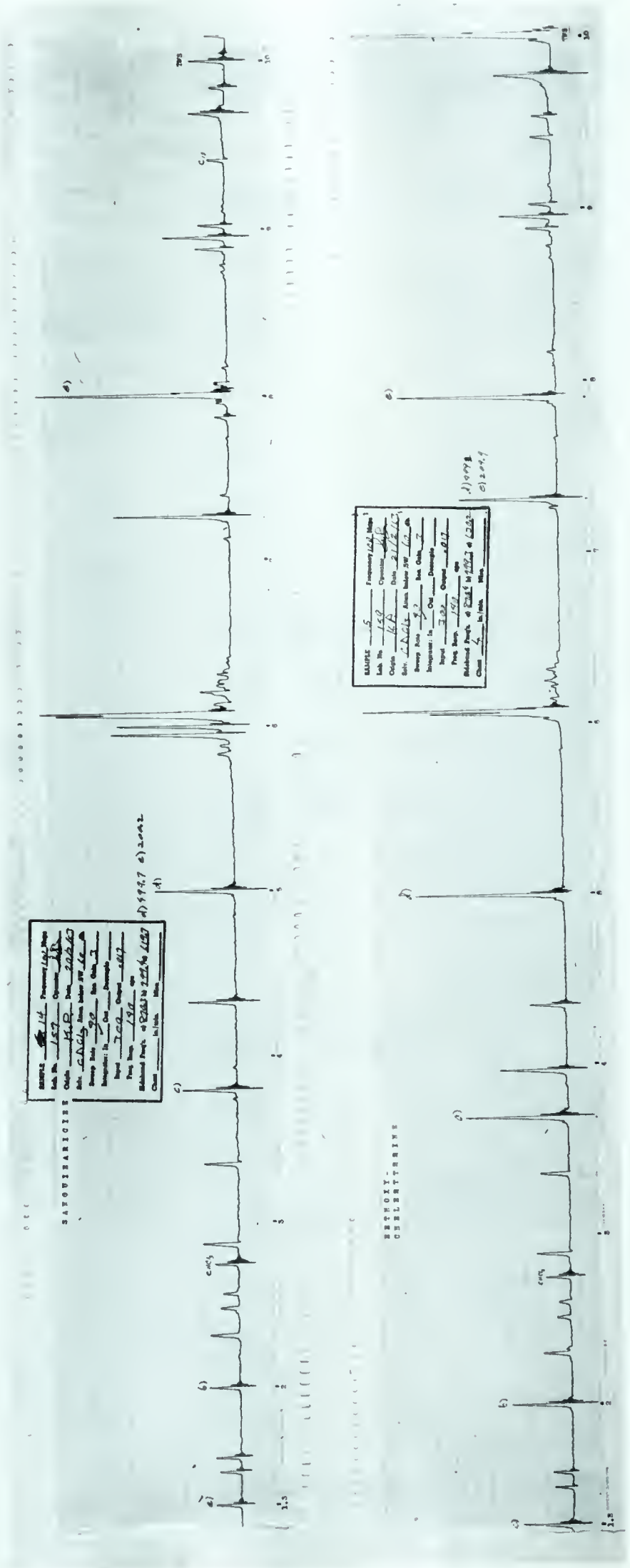


TABLE VII

INFRARED SPECTRAL DATA FOR THE 1,2-BENZOPHENANTHRIDINE ALKALOIDS

FUNCTION	CHELIDONINE	CHELERYTHRINE			
	BASE	BASE	NOR-	OXY-	DIHYDRO-
methylenedioxy	940	935	940	932	940
methoxyl	---	2850	2840	2840	2850
lactam	---	---	---	1660	---
hydroxyl	3620	---	---	---	---

FUNCTION	METHOXYCHELERYTHRINE				SANGUINARINE			
	BASE	NOR-	OXY-	DIHYDRO-	BASE	NOR-	OXY-	DIHYDRO-
methylenedioxy	940	945	940	940	945	940	940	945
methoxyl	2850	2840	2840	2840	---	---	---	---
lactam	---	---	1660	---	---	---	1650	---
hydroxyl	---	---	---	---	---	---	---	---

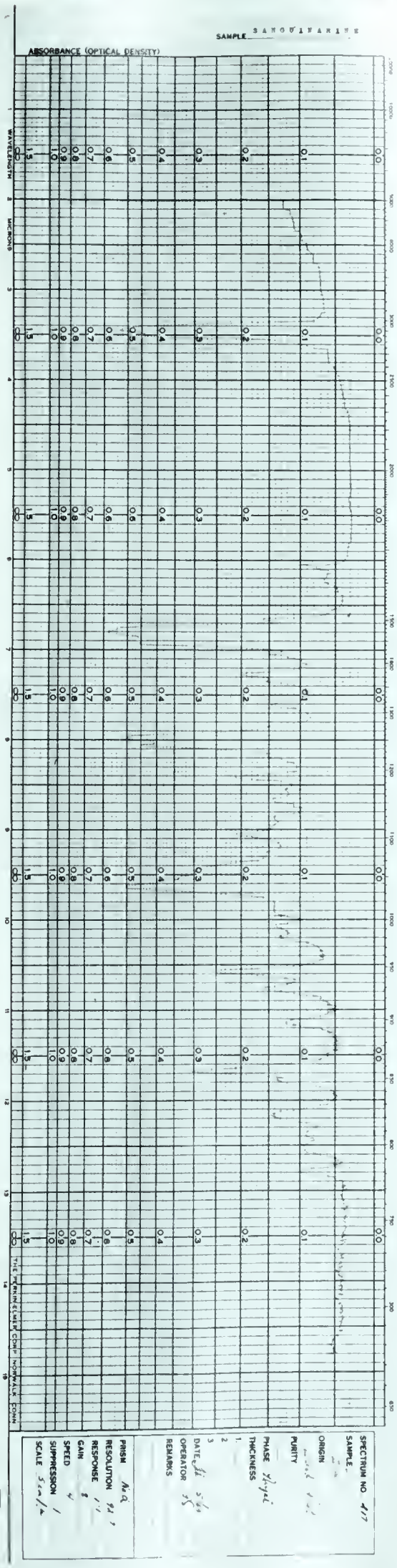
FUNCTION	SANGUINARICINE				XANTHOFAGARINE (36)	
	BASE	NOR-	OXY-	DIHYDRO-	BASE	OXY-
methylenedioxy	---	---	---	---	940	940
methoxyl	2850	2810	2840	2850	2850	2850
lactam	---	---	1625	---	---	1630
hydroxyl	---	---	---	---	---	---

ALL VALUES RECORDED IN RECIPROCAL CENTIMETERS

($cm.^{-1}$)

FIGURE XIII

INFRARED SPECTRA OF THE 1,2-BENZOPHENANTHRIDINE ALKAIOIDS



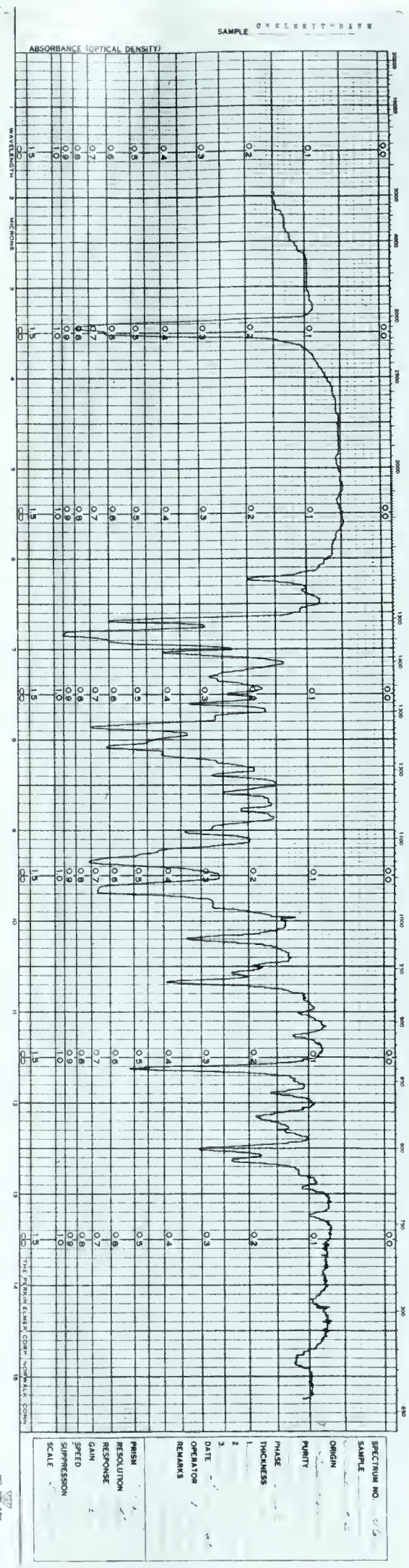
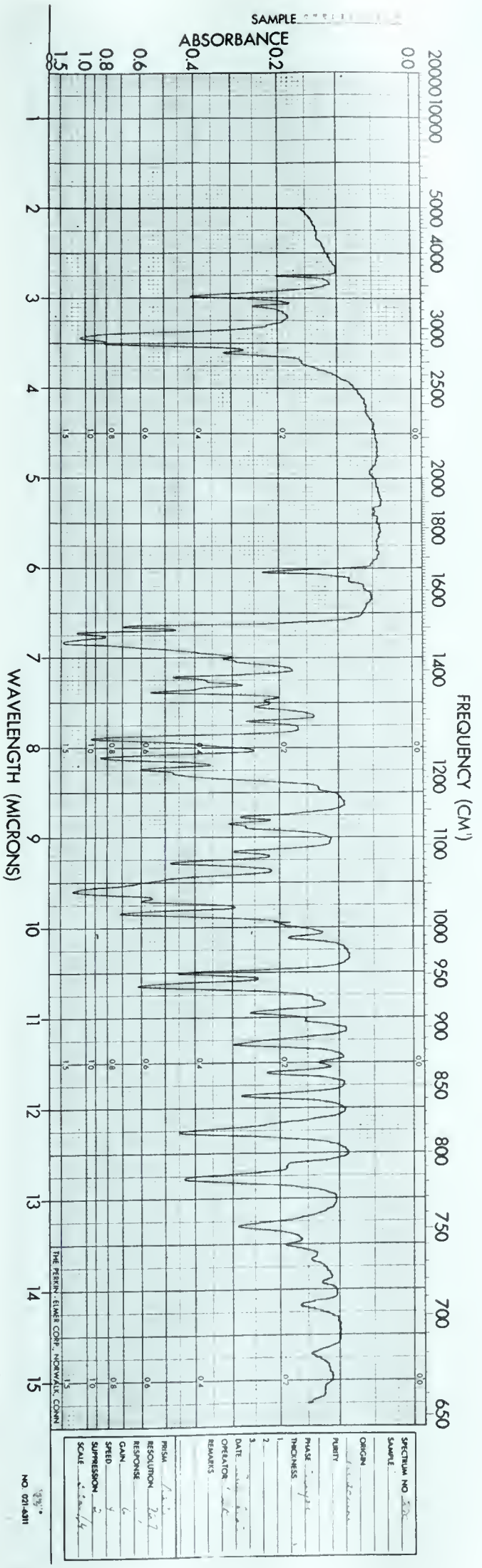


FIGURE XIII CONTINUED



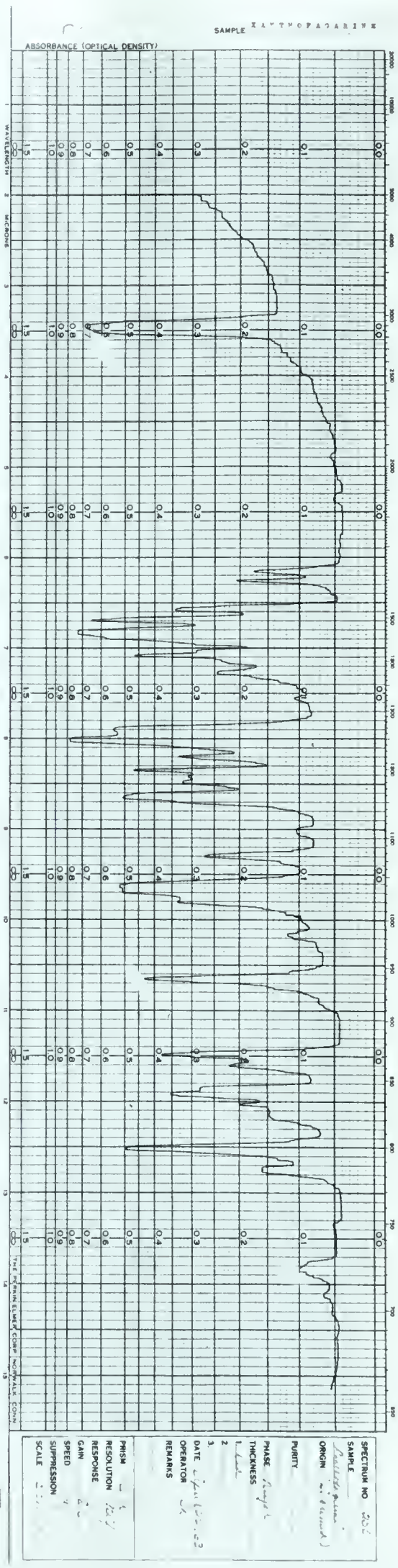
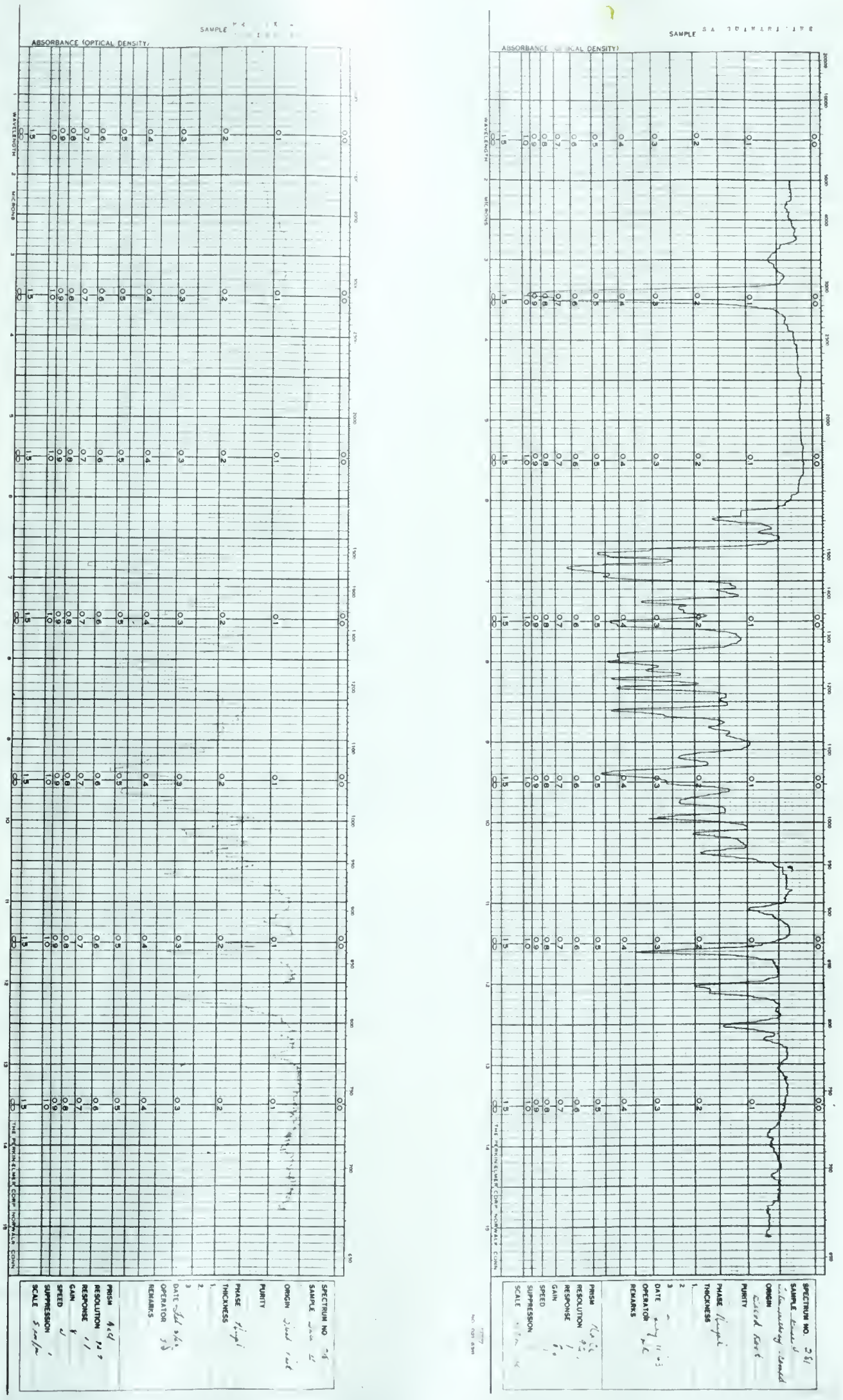


FIGURE XIII CONTINUED



The solution was evaporated to approximately 2 ml. and spotted on a thick plate and 3.4 mg. of material was obtained. Although both oxy-compounds had the same fluorescence, a thin layer chromatogram indicated that the isolated compound was the expected oxysanguinaricine. The Rf values are:

(1)	oxysanguinaricine	0.47
(2)	oxymethoxychelerythrine	0.42
(3)	mixture of (1) and (2)	0.45
(4)	oxysanguinaricine	0.48
(5)	mixture of (1) and (4)	0.47
(6)	mixture of (2) and (4)	0.45

NOTE: The mixtures did not resolve into two spots as expected, however, the spots resulting from the mixtures of (1) and (2), and (2) and (4) were very large, covering the entire region for both compounds. The spot resulting from the mixture of (1) and (4) gave a spot which resembled pure oxysanguinaricine. No depression was found when a mixed melting point was done and the infrared spectra were identical in all respects.

7. ISOLATION AND IDENTIFICATION OF α-ALLOCRYPTOPINE

A deep blue, almost non-fluorescent band occurring near the base line of the plates was removed, extracted from the silicagel and purified by recrystallization from methanol. This material had a m.p. of 161-63° and was found, by means of infrared spectra and mixed melting points, to be identical with our fraction IIIa (FIGURE VII). The infrared spectrum showed that this compound contained a methylenedioxy group(s) (940 $cm.^{-1}$), a methoxyl group(s) (2850 $cm.^{-1}$), a carbonyl group (1650 $cm.^{-1}$). Hence, this alkaloid was suspected of being a protopine alkaloid since they have been reported occurring in this plant and have the above features. A comparison,

by TLC, with authentic samples of α-allocryptopine and cryptopine showed that our alkaloid was most likely α-allocryptopine. This was, in fact proven by their identical infrared spectra, mixed melting point (isolated alkaloid m.p. 161-63°, α-allocryptopine 161-63°, mixed 161-63°) and mixed melting point of the hydrochloride salts (isolated alkaloid 187-90°, α-allocryptopine hydrochloride 190-92, mixed 186-89°).

8. INVESTIGATION OF THE MINOR ALKALOIDAL COMPOUNDS

(a) Base 5

This fraction, which fluoresces as a broad blue band (Rf=0.55), was found to be a mixture of oxysanguinarine and oxychelerythrine. These compounds were separated by rerunning this band on a thick plate. The infrared spectra were identical with those of the synthetic oxycompounds and the mixed melting points with their synthetic counterparts showed no depression.

(b) Base 6

This fraction, which also has a blue fluorescence (Rf=0.48), was similarly found to be a mixture of oxymethoxychelerythrine and oxysanguinaricine.

(c) Base 1.a

This fraction (Rf=0.88) fluoresces only after exposure to the air for sometime, or to ultraviolet light for a short time. When this band does fluoresce its color is nearly that of sanguinarine near the top changing to that of chelerythrine near the bottom. This band, when isolated, was found to be a mixture, mainly of sanguinarine and chelerythrine, with

traces of sanguinaricine and methoxychelerythrine. This led us to believe that the original band was due to a mixture of the dihydro-alkaloids. Hence more plates were run and band 1.a was again removed and the alkaloids isolated only this time the neutral plates were used and the extraction solvent was basified with ammonia. Using this procedure dihydrosanguinarine and dihydrochelerythrine were isolated and by means of a thin plate, dihydrosanguinaricine and dihydromethoxychelerythrine were shown to be present.

Certain preliminary experiments with a limited amount of fresh plant material would indicate that the alkaloids are present in the plant as the dihydro-derivatives which are converted to the unsaturated bases during the normal collection and extraction procedures.

(d) Further chromatographic data shows that traces of the four nor-compounds are also present on the plates, although only in trace amounts. They are found as weakly-fluorescing greenish-yellow spots (sanguinarine Rf=0.65, chelerythrine Rf=0.63, sanguinaricine Rf=0.58 and methoxy-chelerythrine Rf=0.55).

9. CHEMICAL CONFIRMATION OF THE SUBSTITUTION PATTERNS

(a) Attempted synthesis of the degradation acids of methoxy-chelerythrine and sanguinaricine.

(i) Synthesis of Hemipinic acid

560 mg. of opianic acid were dissolved in 60 ml. of water and sufficient sodium hydroxide to yield a solution of pH 10. To this solution, 500 mg. of finely powdered potassium permanganate were slowly added, with constant

stirring, over a period of one-half hour. After addition, the mixture was stirred for a further fifteen minutes, acidified with hydrochloric acid and sulfur dioxide gas was bubbled through the solution until it became colorless. It was then extracted continuously with ether for 48 hours and the ether was evaporated under vacuum to yield 427 mg. of residue. This residue was crystallized from ether and melted at 167-68°. As this is the literature (79) melting point for the anhydride, some of this material was refluxed with acetic anhydride and recrystallized from ether. This anhydride and the reaction product showed no depression of melting points. Therefore, it was concluded that it is the anhydride that is the reaction product. This compound showed the typical anhydride peaks (1850, 1770, 1250 and 1055 $cm.^{-1}$) as well as the methoxyl absorption (2850 $cm.^{-1}$) in the infrared.

(ii) Attempted synthesis of hydrastic acid

(iia) The first attempts to produce hydrastic acid were by oxidation of piperonyl butoxide [α-[2-(2-butoxyethoxy) ethoxy] -4,5-methylenedioxy-2-propyltoluene]. However, the only product identifiable from permanganate oxidation in base or acetone, as well as a nitric acid oxidation was butyric acid.

(iib) 10 g. of piperonal was heated with 7 g. of malonic acid in 100 ml. of pyridine and 2 ml. piperidine on a

steam bath for two hours. This reaction mixture was then concentrated to one-half its volume cooled and filtered. The precipitate was then washed, first with 2% hydrochloric acid and then with water, and dried yielding 10.49 g. of piperonylacrylic acid (4,5-methylenedioxycinnamic acid).

Reduction of the double bond failed with 2% sodium amalgam in ethanol, was very sluggish with palladium on charcoal in ethanol or glacial acetic acid and was slow and incomplete with 2% sodium amalgam in basified water. However, reduction was rapid and complete with lithium aluminum hydride in ether (142). Therefore, 10.3 g. of piperonylacrylic acid was dissolved in 500 ml. of ether and added to 100 ml. of an ether solution containing 0.5 g. of lithium aluminum hydride. After addition of all the unsaturated acid, the mixture was refluxed for two hours, then water was cautiously added to clear the solution of unreacted hydride. Dilute sulfuric acid was added and the reaction mixture was worked up in the usual way to yield 8.47 g. of dihydropiperonylacrylic acid, m.p. 75-80°.

The next step, formylation, proved to be the difficult one of the syntheses and never was successfully overcome. Only one procedure gave an isolatable yield.

A procedure based upon that used by Stevens (145) was used. 5 g. of dihydropiperonylacrylic acid was dissolved in 25 ml. of hot glacial acetic acid and 1 ml.

of hydrochloric acid and 10 ml. of formalin were added. The mixture was heated on a steam bath for one hour, then poured into water, and extracted with chloroform. The chloroform was evaporated after being washed with a sodium carbonate solution. The residue (3 g.) was dissolved in a 20% sodium hydroxide solution and extracted again with chloroform. The aqueous phase is then re-acidified and re-extracted with chloroform, which is evaporated "in vacuo" to yield 1.1 g. of lactone. An infrared spectrum showed lactone absorption at 1735 $cm.^{-1}$ and methylenedioxy absorption (930, 1040, 1360 and 1480 $cm.^{-1}$).

One gram of the lactone was dissolved in 100 ml. of 5% sodium hydroxide solution. To this solution 3 g. of finely powdered potassium permanganate was slowly added, with constant stirring, over a period of two hours. After addtition was complete, the mixture was stirred for a further thirty minutes, acidified with hydrochloric acid and sulfur dioxide gas was bubbled through the solution until it became colorless. It was then extracted continuously with ether for 3 days. The ether was evaporated "in vacuo" to yield 12 mg. of residue. This residue could not be crystallized and consequently the material used for melting point and infrared spectrum was impure. The amorphous material melted at 150-55° and gave an infrared spectrum which showed strong carbonyl absorption (1760 and 1710 $cm.^{-1}$) and acid hydroxyl peaks (2900,

1260, and 935 cm.$^{-1}$). Although the methylenedioxy band is masked by the acid hydroxyl band, evidence for the presence of the methylenedioxy band is found in the peaks at 1480 1400, 1200 and 1040 cm.$^{-1}$.

Another procedure which was essentially the same as above, boron trifluoride in ether being used instead of hydrochloric acid, was used but no lactone was isolated.

The third procedure parallels one found in Organic Synthesis (153). Piperonal was converted to the alcohol by action of sodium borohydride in methanol. One gram of this alcohol dissolved in chloroform was slowly added to a previously prepared mixture of 0.75 g. of N-methylformanilide and 0.85 g. of phosphorous oxychloride, so as to keep the temperature below 35°. After addition is complete the mixture is allowed to stand for two hours. The viscous liquid is then poured into a vigorously stirred mixture of 50 g. of ice and 50 ml. of water. The aqueous layer is separated and extracted with three 50 ml. portions of chloroform. The extracts are combined with the organic layer and washed twice with 50 ml. portions of dilute hydrochloric acid to remove all traces of N-methylaniline. The combined chloroform extracts are washed twice with a saturated sodium bicarbonate solution, then with 15 ml. of water, and finally dried over anhydrous sodium sulfate. Evaporation of the chloroform yielded approximately 250 mg. of compound that was neither lactone nor starting material,

but what was thought to be 3-phenyl-(4,5-methylenedioxy)-n-propanal.

10. OXIDATIVE DEGREDATION OF THE 1,2-BENZOPHENANTHRIDINE ALKALOIDS.

(a) Chelidonine

70 mg. of chelidonine was dissolved in 5 ml. of 2N sulfuric acid and diluted to 10 ml. with water. A saturated solution of sodium carbonate was then added dropwise until the solution became turbid. The solution was then agitated by a vibromixer and 1.0 g. of finely powdered potassium permanganate was slowly added over a period of one hour. Agitation was continued for a further one-half hour, then the solution was acidified, decolorized with sulfur dioxide and continuously extracted with ether for three days. Evaporation of the ether yielded 92 mg. of a mixture of acids and other organic materials. This residue was dissolved in dilute ammonia and calcium chloride solution to precipitate the oxalic acid as calcium oxalate. The filtered solution was acidified with hydrochloric acid and re-extracted with ether. This time only 14.4 mg. of residue was obtained upon evaporation of the ether. This residue, however, when spotted on a neutral silicagel plate and developed in ethanol:ammomia:water (18:2.5:2.5) showed two acidic spots (Rf=0.05 and 0.08) when sprayed with bromothymol blue. One of these compared to the synthetic hydrastic acid (Rf=0.05) and the other is presumably 3,4-methylenedioxyphthalic acid.

(b) Sanguinaricine

107 mg. of sanguinaricine was dissolved in 2 ml. of hot, 2N sulfuric acid and then diluted to 5 ml. with water. A saturated solution of sodium carbonate was added until the solution became turbid. The solution was agitated with a vibromixer and 0.6 g. of finely powdered potassium

permanganate was added over a period of one hour. The mixture was then worked up similarly to chelidonine and 7.2 mg. of amorphous material was obtained. Thin layer chromatography showed two acidic spots, one of which appeared to be hemipinic acid (Rf=0.19; synthetic hemipinic, Rf=0.20). The other is presumably m-hemipinic acid (Rf=0.52).

(c) Methoxychelerythrine

43 mg. of methoxychelerythrine were treated similarly to sanguinaricine and 2.4 mg. of residue were obtained. Thin layer chromatography showed two acidic spots, one of which was hemipinic acid (Rf=0.20). The second spot compared (Rf=0.05) with the synthetic hydrastic acid and with the one spot from chelidonine and is therefore presumably hydrastic acid.

PART VI

APPENDIX

A. SPRAY REAGENTS

1. DRAGENDORFF'S REAGENT (MODIFIED) (148)

Bismuth Subnitrate	3.4 g.
Glacial Acetic Acid	20.0 ml.
Potassium Iodide	10.0 g.
Distilled Water	60.0 ml.

Dissolve the potassium iodide in 10 ml. of water and add the glacial acetic acid. The bismuth subnitrate is then added in portions. The resultant solution is filtered and water is added to make 60 ml.

For use the above solution is diluted as follows:

Dragendorff's Reagent Concentrate	1 ml.
Glacial Acetic Acid	3 ml.
Water	6 ml.

2. MAYER'S REAGENT

Mercuric Chloride	1.36 g.
Potassium Iodide	5.0 g.
Distilled Water	100.0 ml.

Dissolve the mercuric chloride in 60 ml. of water and the potassium iodide in 10 ml. of water. Slowly add the potassium iodide solution to the mercuric chloride solution while rapidly stirring. Add distilled water to give 100 ml.

A white or cream precipitate is a positive test for alkaloids when Mayer's reagent is added to an acidic solution of the alkaloid.

3. CERIC SULFATE SPRAY

Ceric Sulfate	5 g.
Sulfuric Acid	10 ml.
Distilled Water to	100 ml.

Dissolve the ceric sulfate in the 10% sulfuric acid and use as is.

4. BROMOTHYMOL BLUE SOLUTION B.P. 1958

Bromothymol Blue	0.1 g.
N/20 Sodium Hydroxide	3.2 ml.
Alcohol (90%)	5.0 ml.
Alcohol (20%) to	250.0 ml.

Warm the bromothymol blue with the N/20 sodium hydroxide and add the 90% alcohol. After solution is effected, add suficient alcohol (20%) to produce 250 ml.

B. TLC SLURRIES

1. 8 cm. x 10 cm. PLATES FOR QUALITATIVE WORK

(a) SILICAGEL G

Silicagel G	25 g.
1,2-Dimethoxyethane	10 ml.
Distilled water	40 ml.

The silicagel is triturated with the liquid phase until a homogenous slurry is formed. The slurry is then poured into an applicator and the plates spread. The above figures are for 18 plates 0.25 mm. thick.

(b) ALUMINA

Alumina	25 g.
Distilled Water	30 ml.

The slurry and plates are prepared in the same manner as the silicagel plates. The above figures are also for 18 plates 0.25 mm. thick.

2. 20 cm. x 20 cm. PREPARATIVE PLATES

Silicagel G	50 g.
1,2-Dimethoxyethane	20 ml.
Distilled water	80 ml.

These amounts, when mixed like the 8 cm. x 10 cm. plates, will give 2 plates 1.0 mm. thick.

3. NON-ACIDIC PLATES

When preparing silicagel plates for detecting acidic materials, prepare as above only replace the distilled water with 0.1N sodium carbonate solution. Spray indicators, such as bromothymol blue, will then be useful. The use of 0.1N sodium carbonate was used instead of 0.1N sodium hydroxide (146) because it was found to give plates of better stability. This modification in preparing the slurry did not greatly alter the separation of, or Rf values of the components.

C. TLC SOLVENTS

1.	Chloroform	8 ml.
	n-Hexane	12 ml.
	Methanol	2 ml.

Most compounds give some form of separation with this solvent. It was used for all preparative plates.

2.	Chloroform	6 ml.
	n-Hexane	12 ml.
	Diethylamine	2 ml.

This is unsatisfactory for neutral plates because diethylamine interferes with the indicator sprays, however it is a helpful solvent when running salts of acids or bases.

3.	Ethanol (95%)	18.0 ml.
	Ammonium Hydroxide	2.5 ml.
	Distilled Water	2.5 ml.

This is a good solvent for acidic materials, especially those related to the phthalic acids.

4. Cyclohexane 9 ml.
Chloroform 9 ml.
Glacial Acetic Acid 2 ml.

This is a good solvent for the separation of quaternary alkaloids when used with alumina plates (154).

5. Chloroform 10 ml.
Ethyl acetate 10 ml.
Methanol 2 ml.

This is a good solvent for separation of the protopine alkaloids (148).

D. 1. SODIUM AMALGAM (155)

A 2% amalgam was prepared by placing 6.9 g. of sodium in a 250 ml. round bottom flask fitted with a separatory funnel containing 340 g. of mercury. The flask is flushed out with nitrogen and about 10 ml. of mercury is added to the sodium and the flask is warmed slightly with a free flame until the reaction starts. Once this point is reached the reaction can be kept in progress by slow addition of mercury until the total amount has been added. At the end, the still hot, molten amalgam is poured onto a piece of Transite board. The amalgam is allowed to set and cool before it is ground to a powder and transferred to a tightly stoppered bottle.

2. PREPARATION OF DIAZOMETHANE IN TETRAHYDROFURAN

The method described below is a modification of the method found in Organic Synthesis (156).

PROCEDURE

A 125 ml. distilling flask is fitted with a condensor set for distillation and with a long-stem-capillary dropping funnel. The condensor is connected by means of an adapter to a 250 ml. erlenmeyer flask. Through

a second hole in the stopper in the erlenmeyer is placed an outlet tube bent so as to pass into and nearly to the bottom of a second erlenmeyer flask (125 ml.) which is not corked. Both flasks are cooled in a salt-ice-bath. The first erlenmeyer flask contains 20 ml. of THF and the second contains 25 ml. of THF. The inlet tube passes below the surface of the THF in the second flask.

In the distilling flask, 10 g. of potassium hydroxide are dissolved in 15 ml. of water and 35 ml. of carbitol (b.p. 192-202°) and 10 ml. THF are added. (Although the carbitol is not essential in this procedure it was found to enhanse the reaction.) The dropping funnel is attached and adjusted so that the stem is just above the surface of the solution in the distilling flask.

A solution of 21.5 g. of p-tolylsulfonylmethylnitrosamide (Diazald) in 50 ml. of THF. The distilling flask is heated in a water bath at 95-100°C. and the nitrosamide solution is added at a regular rate during 15-20 minutes. Agitation during the addition of the nitrosamide solution is not necessary but it does speed the rate somewhat. (Caution: start with gentle agitation to avoid an overly vigorous reaction and possible explosion.) As soon as the nitrosamide solution has been added, additional THF (25-50 ml.) is placed in the dropping funnel and added as above until the distillate is colorless.

The yellow distillate contains 2.7-2.9 g. of diazomethane and is stable for up to four days when kept in the freezing compartment of a refrigerator at 0° F.

PART VII

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